

Thannhauser's TEXTBOOK OF
METABOLISM AND
METABOLIC DISORDERS

VOLUME I

Edited by NEPOMUK ZOLLNER

American Edition Translated and Edited by
SOLOMON ESTREN

CFTRI-MYSORE



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1. obesity ② malnutrition
3. undernutrition ④ ~~prostate~~ enzymes
5. biological oxidation
6. thyroid gland
7. " disorders.
8. carbohydrate metabolism
9. diabetes 10. insulin
11. protein metabolism
12. amino acids "

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THANNHAUSER'S TEXTBOOK OF METABOLISM
AND
METABOLIC DISORDERS

CHAPTERS IN VOLUME I:

- I. General Metabolism*—Nepomuk Zöllner
- II. Intermediate Metabolism*—Benno Hess

Carbohydrate Metabolism:

- III. Biochemistry of Diabetes*—Joachim Kühnau and Claus von Holt
- IV. Clinical Section*—Ferdinand Bertram

Metabolism of Proteins and Amino Acids:

- V. Theoretical Section*—Kurt Felix
- VI. Clinical Section*—Jan Waldenström

CHAPTERS IN VOLUME II:

- VII. Metabolism of Mucopolysaccharides*—Josef Fellig
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- IX. Metabolism of Neutral Fats and Fatty Acids*—Nepomuk Zöllner
- X. Metabolism of Lipids*—Nepomuk Zöllner
- XI. Metabolism of the Steroids and Carotinoids*—Nepomuk Zöllner
- XII. Metabolism of the Bile Acids*—Pierre Fallot
- XIII. Metabolism of the Blood and Bile Pigments*—Pierre Fallot
- XIV. The Coagulation of Blood*—Mario Stefanini
- XV. Metabolism of Iron*—Myron Pollycove
- XVI. Metabolism of Calcium and Phosphorus*—Heinz Hungerland
- XVII. Metabolism of Water and Electrolytes*—Hanns C. Moll and Guy W. Daugherty

Thannhauser's Textbook of Metabolism and Metabolic Disorders

SECOND EDITION

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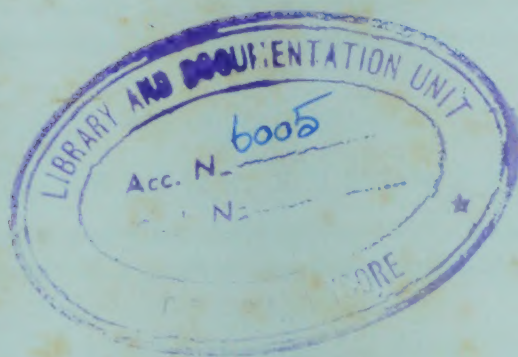
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Preface to the American Edition of Metabolism and Metabolic Disorders

GIOVANNI BATTISTA MORGAGNI demonstrated in 1761 in his famous book *De Sedi-bus et Causis Morborum*, that anatomical changes of organs are the cause of many diseases. In the first edition of this book, published in Munich in 1929, it was emphasized that the cause of disease in man may also be functional disorders, inborn or acquired, of chemical or enzymatic nature. This basic idea has led, in the last thirty years, to such an intensification of research on intermediary metabolism that it has almost choked its purpose. It has become more and more difficult for a single person to master the complexity of the pertinent clinical, chemical and enzymatic literature.

In 1934 the author was forced to interrupt his clinical and chemical studies of metabolic diseases by the political upheaval in Germany. A new edition of the original book was not possible for a long time.

DR. NEPOMUK ZÖLLNER, working for two years with the author at the Pratt Clinic – New England Center Hospital and in the research laboratories of the Boston Dispensary, took on the difficult task of rewriting the book, together with fifteen other authors of various countries, each having special knowledge of his topic. I left the rewriting of the book entirely to these authors, having in mind that a younger generation should continue, in the future, the tradition of the book. Tradition is the fundament of knowledge.

The new German edition was published in 1957 by Georg Thieme, Stuttgart. Dr. SOLOMON ESTREN deserves my sincere gratitude in accomplishing the American edition, which is being published by Grune and Stratton, Inc., New York, 1962. It is with great satisfaction on my part that the English edition of *Metabolism and Metabolic Diseases* is offered to the medical profession of America.

Boston, October 1961

SIEGFRIED J. THANNHAUSER, M.D., Ph.D.

Preface to German Edition

Rhythm was the beginning of music.
Creative thought was the beginning of investigation.

In 1929, at the time of the first edition of this book, biochemistry had largely attained the goal of determining the constituents of organic matter. With justified pride the investigators had correctly put together the many small stones which form the mosaic of organic nature. In addition the synthesis *in vitro* of the natural building stones was accomplished in the laboratories.

With the passage of time, however, this static, well-established mosaic suddenly became alive and dynamic. Stimulated originally by the fundamental methodology and basic research of OTTO WARBURG, investigators throughout the world began to study the details of the mechanisms of enzymatic reactions. The last thirty years have comprised a new, advanced epoch in biochemistry.

The mechanism of oxidation and reduction in metabolic processes was explained. The chemical structure of the various coenzymes and their role in anabolism and catabolism in the processes of intermediate metabolism were elucidated. The introduction of isotopic technics allowed many investigators, especially in the United States, to uncover and clarify many of the intermediate reactions of synthesis and catabolism within the cell (cellular metabolism). The dynamics of intermediate metabolism, once merely a rhetorical phrase, became accessible to investigation. The volume of work became so great that it became more and more difficult to read the profuse literature critically. For this reason I have been reluctant to attempt a new edition of what must be an authoritative treatise on metabolism by myself.

It is gratifying to me, therefore, that my former colleague and my friend, Dr. NEPOMUK ZÖLLNER, has undertaken to revise my book with the aid of experts throughout the world. I myself have declined to write a chapter: let a new, younger generation express its own thoughts in writing. The various authors have studied both the old and the new literature critically and have interpreted it for the reader. The authors have re-examined old concepts and observations and have devised modern concepts of their own. Among other things, in many places this book attempts to clarify many of the complicated phenomena of metabolism. Unfortunately, the physician is often exposed to a sort of intellectual dishonesty in that metabolic phenomena are merely given a new name without any concomitant better explanation of the mechanism of the processes. It was – and is – the purpose of this book to explain such phenomena and to instill and perpetuate scientific thinking as the central point of the art of medicine.

“Knowledge is proud that he has learned so much;
Wisdom is humble that he knows no more.”

William Cowper

Boston, January 1957

S. J. THANNHAUSER

Foreword to German Edition

The first edition of Thannhauser's *Textbook of Metabolism and Metabolic Diseases* was published in 1929. The events of the following years made a second edition impossible, and it was not until some years after the second World War that a second, revised edition could be considered. It soon became evident that it was no longer possible for any single individual to treat the entire field of metabolism and metabolic diseases with the basic and detailed thoroughness necessary for an authoritative work. Fortunately, a number of well-known experts in the various fields of metabolism, most of them friends or students of Dr. THANNHAUSER, were more than willing to contribute to such a work.

Since each investigator approaches the various topics in metabolism from an individual point of view, I had some misgivings that the original nature of each chapter might result in some weakness of the book as a whole. However, although many chapters are entities unto themselves, and although a few topics are discussed in different chapters by different authors, I believe that this volume encompasses the basic concepts of metabolism in logical, unified form. If this new edition, because of the multiplicity of authors, no longer offers the unified, single concept of the first edition, it nonetheless covers the basic principles of scientific metabolic investigation and thoroughly details results and applications to clinical practice.

This work has been planned to cover not merely the individual diseases of metabolism, but also all other metabolic disturbances seen in clinical practice. The chapters are classified according to the metabolic systems. Thus, for example, deficiencies of the vitamins niacin, riboflavin, and thiamin are discussed under biological oxidations rather than merely in a clinical chapter on avitaminoses which would lack fundamental metabolic logic.

The chapters written by myself are based largely on work done during my stay in Dr. THANNHAUSER'S clinic in Boston. Dr. THANNHAUSER was good enough to permit me to study his personal notes of the first edition, and I have borrowed liberally from these notes in writing the historical sections. Dr. THANNHAUSER read and criticized my chapters, and I am deeply indebted to him for his help, his stimulating criticism, and his continuing encouragement.

I also wish to thank my chief, Professor Dr. WALTER SEITZ, for the patience and understanding he has shown while the book was in preparation. Special thanks are due to my secretary, Miss ELISABETH ROTHEMUND, without whose constant efforts my labors would have been multiplied many times. My thanks are also offered to my associates Dr. GERTRUD BRAUN, Dr. STEFAN ERNST, and Dr. ERWIN KÖNIG; and to many other friends and colleagues for their help and encouragement.

In Germany, much has been written in recent years about a "crisis of academic medicine." There is no such crisis, however, but rather, if anything, of practicing physicians to translate the data of academic medicine into clinical practice. It is not necessary to reform medicine but rather to improve communication and education.

It is well known that the great teachers of medicine have at the same time been great clinicians. We honor one of these great men in this work which bears his name. An even truer honor, however, would be for us to emulate his human as well as his medical dedication, in the spirit of "a blend of humanistic goodness and scientific wisdom."

Munich, March 1957

NEPOMUK ZÖLLNER

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Abbreviations

ADP	adenosine diphosphoric acid	n	number of electrons
AHG	antihemophilic globulin	NMN	nicotinamide nucleotide
AMP	adenosine monophosphoric acid (= adenylic acid)	$\sim P$	high-energy phosphate bond
ATP	adenosine triphosphoric acid	P	orthophosphate (orthophosphoric acid)
B	base (a cation)	PP	pyrophosphate (pyrophosphoric acid)
CDP	cytidine diphosphoric acid	PRPP	phospho-ribosyl-pyrophosphate
CMP	cytidine monophosphoric acid (= cytidylic acid)	PTA	plasma thromboplastin ante- cedent
CoA	coenzyme A	PTC	plasma thromboplastin compo- nent
CTP	cytidine triphosphoric acid	PTF	plasma thromboplastic factor
DNA	deoxyribonucleic acid	Q_{O_2}	oxygen utilization in mm ³ per mg dry weight per hour
ΔE_0	potential difference in volts	$Q_{ATP}^{O_2}$	synthesis of ATP under oxygen
$\Delta E'_0$	potential difference in volts at pH 7	$Q_{ATP}^{N_2}$	synthesis of ATP under nitrogen
ΔF	change in free energy	$Q_M^{N_2}$	synthesis of lactic acid under nitrogen
ΔH	heat change at constant pressure	RNA	ribonucleic acid
ΔU	change in internal energy	$\sim S$	high-energy sulfur bond
DH-cycle	Dickens-Horecker cycle	SF	stable factor of blood coagulation
DOCA	desoxycorticosterone acetate	SPCA	serum prothrombin conversion accelerator
DPN ⁺ or DPN	diphosphopyridine nucleotide = coenzyme I (oxidized form)	STH	somatotrophic hormone of the anterior pituitary (growth hor- mone)
DPNH or DPNH ₂	diphosphopyridine nucleotide = coenzyme I (reduced form)	TCC	thromboplastic cellular compo- nent
e	electron	TPP	thiamin pyrophosphate
E_0	normal potential in volts	ΔS	change in entropy
E-M path- way	Embden-Meyerhof pathway of catabolism	TPC	thromboplastic plasma compo- nent
F	Faraday	TPN ⁺ or TPN	triphosphopyridine nucleotide, coenzyme II (oxidized form)
FAD ⁺ or FAD	flavin adenine dinucleotide (oxidized form)	TPNH or TPNH ₂	triphosphopyridine nucleotide, coenzyme II (reduced form)
FADH or FADH ₂	flavin adenine dinucleotide (reduced form)	TSH	thyrotropic hormone of the ante- rior pituitary
FMN	flavine mononucleotide	UDP	uridine diphosphoric acid
GDP	guanosine diphosphoric acid	UDPG	uridine diphosphate glucose
GMP	guanosine monophosphoric acid (= guanylic acid)	UMP	uridine monophosphoric acid (= uridylic acid)
GTP	guanosine triphosphoric acid	UTP	uridine triphosphoric acid
IDP	inosine diphosphoric acid		
IMP	inosine monophosphoric acid (= inosinic acid)		
ITP	inosine triphosphoric acid		
K_m	Michaelis constant		
LF	labile factor of blood coagulation		

CHAPTER I.

General Metabolism

By Nepomuk Zöllner

History of Metabolic Research

The ancients believed that all substances in nature, organic as well as inorganic, are composed of the same four basic materials, water, fire, air, and earth. Even man, the most highly developed organism in nature, was thought to be made up of these four substances. Hippocrates (460–364 B. C.) was the first to propose this hypothesis, which was later firmly established by Galen (131–200 A. D.). Galen's four body "humors" symbolized the four elements of nature.

Blood, he said, is moist and warm, like air. Mucus is moist and cold, like water. Yellow bile is dry and warm, like fire. "Black bile" is dry and cold, like earth. When these humors are present within the body in correct proportions and in proper distribution, the body is healthy. When these requirements are not fulfilled, so that one of the humors predominates in some part of the body, a false mixture results – "dyscrasia." "Dyscrasia" was considered to be the cause of illness. The ancient Greeks knew, of course, that the body is composed of flesh, blood, bones, and skin. They must have recognized, however, that simple anatomic considerations were incapable of explaining the functioning of the living body, and that other, physiological considerations must be of importance. The concept of "dyscrasia" as the genesis of disease is the original, first concept of humoral pathology: each disease was considered the result of faulty changes in the body humors. Although, with time, the diagnosis of disease has undergone extensive advances, Galen's basic concept of "dyscrasia" is, in a certain sense, the classic explanation of those disorders which today are classified as "Diseases of Metabolism."

Neither the Greeks nor the Romans made any substantial contributions with regard to the metabolic processes which go on within the body or the purpose and fate of ingested food. They searched for explanations based on logic and philosophy, but failed to make any actual experiments. For almost 1500 years people were satisfied with isolated observations and with purely theoretical concepts. It was not until the Renaissance that, once again, there was a revival of interest in investigation into the phenomena of life. Anatomy developed first and then, in its footsteps, physiology, and the surprisingly modern concept of "dyscrasia" outlined above was relegated to the background. The theoretical approach of the previous centuries was succeeded by experimental approaches to metabolic research.

At the beginning of the Renaissance, Leonardo da Vinci (1452–1519) discovered that an animal could live only in an atmosphere which could also support a flame, but this discovery lay forgotten for 300 years. The seat of medicine at that time lay in the universities of northern Italy. In Padua, at the turn of the sixteenth century, Sanc-

Sanctorius was concerned with the problem of why the weight of ingested food is greater than the weight of excreted feces and urine. A well-known woodcut perpetuates the research of Sanctorius, the first metabolic research performed in man: A scientist is depicted as sitting on a chair which is suspended on a large, one-armed balance. Sanctorius carefully determined the weight of the ingested food and found that his weight during digestion was less than the sum of his initial weight plus the weight of the ingested food. Therefore, some material which had weight must have been excreted unobserved from the body. Sanctorius called this invisible excretion "insensible perspiration" and stressed it in his major work, "*De Medicina Statica Aphorismi*."

In the 17th century, leadership in medical science passed from Italy to France, England, and Holland. Paris saw the rise of an academy of sciences under the sponsorship of Louis XIV and a similar organization, the Royal Society, was founded in London. Nicholas le Fèvre made the observation that metals became heavier as they become red hot and postulated that the increase in weight was due to the absorption of a gas which was also essential for animal respiration. Van Helmont (1577-1644) described a new gas in the presence of which life became impossible: carbon dioxide. Robert Boyle (1621-1679) showed that both life itself and the burning of a flame were impossible in the reduced pressure of a glass jar. John Mayow (1640-1679) expanded these ideas and wrote, in a paper on respiration, "During respiration and during the burning of a flame, air loses some of its elastic strength. It must be concluded that the living organism absorbs some of the same material from air as does a burning flame." Mayow's teachings were not carried further but were overshadowed by a new hypothesis of the phenomena of combustion, the phlogiston theory. It was Stahl (1660-1734), physician to King Frederick William I of Prussia, who proposed the hypothesis that all inflammable substances contain a substance called "phlogiston" which is necessary for the process of combustion. The fruitless search for phlogiston in air led instead to the discovery of oxygen, hydrogen, and nitrogen.

Cavendish (1731-1810) believed that he had discovered phlogiston and called the new gas "inflammable air." Today this gas is known to be hydrogen. Priestley (1733-1804) allowed a candle to burn itself out in a small sealed container and then placed a growing green plant into the container. After a few days, he found that a candle could again burn within the container. In a further experiment, he showed that when yellow oxide of mercury was heated to red heat, a gas was formed which supported the burning of a candle. Priestley concluded that both the growing plant and the red hot mercuric oxide liberated a gas which he called "dephlogisticated air" - i. e., today's oxygen. Cavendish observed that an electrical spark could cause the combination of 2 parts of "inflammable air" with one part of "dephlogisticated air" to give water. Finally, Rutherford (1749-1819) found that when a candle burns almost to the point of extinction in a small closed container, alkali will take up the "fixed air" (carbon dioxide), leaving behind a gas which promptly extinguishes the flame. He called this gas "residual air," and it was later shown to be nitrogen. These important discoveries were the first steps towards a greater understanding of the phenomena of nature. The greatest discoveries, however, were reserved to the genius of Lavoisier, the greatest chemist of the 18th century.

Lavoisier (1743-1794) was the first to show that carbon dioxide ("fixed air") is a compound of carbon and oxygen. When yellow oxide of mercury is heated by itself, oxygen is given off; when it is heated in the presence of carbon, the result is carbon dioxide (Black 1728-1799; Priestley). Lavoisier [1] locked a sparrow in a glass jar and showed that the oxygen within the jar disappears and is replaced by carbon

dioxide. This observation led to basic investigations into the physiology of nutrition by Lavoisier and Laplace in 1780 [2]. These workers determined the amount of carbon dioxide ("fixed air") which was produced in ten hours by a guinea pig. Another guinea pig was placed into a specially constructed ice calorimeter and the amount of ice melted in ten hours was calculated. The authors then determined how much carbon had to be burned in order to give the same amount of carbon dioxide as the guinea pigs, and this amount of carbon was burned in the calorimeter and the amount of ice melted likewise measured. In the animal experiment, it was found that 224 grains of carbon dioxide were produced and 13 ounces of ice melted. The burning of the corresponding amount of carbon resulted in the formation of 224 grains of carbon dioxide and the melting of 10.4 ounces of ice. Lavoisier himself recognized the inherent errors of such experiments: the extremities of the guinea pigs became frozen, the water of respiration was added to that due to the melting of the ice, and the cold itself caused an increased production of heat by the animal. Despite these sources of error, Lavoisier was able to formulate the basic conclusion of his experiment: "The two types of heat – animal heat and the heat produced by burning coal – have nearly the same effect. We may immediately conclude that animal heat is produced largely by the conversion of oxygen ('air pur') into carbon dioxide ('air fixe')." At almost the same time, Crawford in England (1748–1795) showed that for a given amount of oxygen used up in a calorimeter, the temperature of the water in the calorimeter rises to the same extent whether the oxygen was consumed by a burning candle, by burning coal, or by a guinea pig living within the calorimeter.

In further experiments Lavoisier determined the composition of water, potassium nitrate, and sulfuric acid. His precise experimental methods completely shattered the phlogiston theory. In spite of this fact, however, the great chemists of the period – Priestley, Cavendish, and Scheele – continued to uphold the phlogiston theory, and only Black was converted to Lavoisier's hypotheses.

Lavoisier reached his greatest accomplishment in the first experiments in respiration, which he performed in collaboration with Seguin. Seguin's contribution was to devise the gas analyses necessary in experiments on respiration. Unfortunately, there are no descriptions of the apparatus which Lavoisier used. Only a few pictures are available, made by Lavoisier's wife and the well known artist Gérard David from memory after Lavoisier's death. These drawings have more artistic than scientific value. However, although his method is not available to us, Lavoisier's conclusions are:

1. The oxygen consumption of a resting, fasting man at 26° C is 24 liters per hour.
2. At a temperature of 12° C, the oxygen consumption rises to 27 liters per hour.
3. Following a meal, the oxygen consumption is 36 to 38 liters per hour.
4. When, in addition, the subject does physical work, the oxygen consumption rises to 65 to 91 liters per hour.

Lavoisier thus found that the amount of oxygen consumed and the amount of carbon dioxide produced depends on the environmental temperature, food, and work. Soon after he had discovered the phenomena of oxidation and analyzed them chemically, Lavoisier turned his attention to other problems of life, and stated his thesis that "life is a chemical process." Lavoisier died by the guillotine. As he was beheaded, the famous mathematician Lagrange whispered to his neighbor, "His head has fallen in the wink of an eye. A hundred years will not suffice to produce another such head."

Lavoisier believed that, within the body, the process of oxidation takes place in the lungs. Here, he felt, a fluid containing hydrogen and carbon was oxidized by inspired

oxygen to form water and carbon dioxide. Lavoisier's friend Lagrange argued against this concept because if it were so, he said, the temperature of the lungs must be higher than anywhere else in the body. After Magnus (1802–1870) had shown the presence of oxygen and carbonic acid in the blood, the blood was considered to be the site of the process of oxidation until it became known that the blood functioned in this respect only as a transport organ.

With Justus von Liebig (1803–1873) began a new era of research into metabolism. Liebig studied in Paris at the same time as LaPlace, Berthelot, Gay-Lussac, and Magendie. At the age of 21, Liebig was appointed to the newly created chair of chemistry in Giessen, where he laid the foundations of modern chemistry by elementary analyses of organic compounds. Many organic compounds had long been known, but their basic composition and synthesis became well known only after Liebig had developed new techniques of study. Liebig was the first to divide foodstuffs into proteins, carbohydrates, and fats. He showed that it was not carbon and hydrogen which are oxidized within the body, but the high-molecular foodstuffs themselves. The nitrogen content of proteins was known to Liebig through his studies, and he made the suggestion that the amount of nitrogen in the urine can be used as a measure of the protein metabolism of the animal body.

Bidder and Schmidt [4] took up this concept of Liebig and were the first to subject it to experimental verification in animals. The experiments of Bidder and Schmidt were extended and fully confirmed by the extensive studies of Carl von Voit (1831 to 1908). Voit [5] fed a dog for 25 days with 29 kg of meat (60 pounds), and made the following observations:

Amount of meat ingested	29 kg = 986.0 grams N
Amount of nitrogen in urine	943.7 grams N
Amount of nitrogen in feces	39.1 grams N
Total nitrogen excreted	982.8 grams N
Difference between intake and output	3.2 grams N (0.3%)

Thus, almost all the nitrogen ingested in the food was excreted in the urine. Nitrogen intake is entirely by way of food, and nitrogen excretion is almost entirely in the urine. As Voit showed, the nitrogen of the air is dissolved in the body fluids in proportion to its partial pressure, but it does not enter into metabolic processes within the body. In the breakdown of protein, similarly, nitrogen gas is not formed. The ammonia which is produced by catabolism of protein does not appear in the exhaled air, which, as a matter of fact, contains no compounds of nitrogen derived from nitrogen metabolism. Very small amounts of nitrogen-containing compounds are present in the sweat, in desquamated skin, and in the hair.

The close relationship between protein metabolism and nitrogen excretion stimulated Voit and Pettenkofer to construct an apparatus to measure the excretion of carbon dioxide and water from the body. The amount of carbon dioxide would then permit the calculation of the amount of carbon-containing material which was oxidized. Lavoisier, Regnault, and Reiset had already built respiratory apparatus, but the details of the apparatus used by Lavoisier were unknown, and the glass jar apparatus of Regnault and Reiset for experiments on small animals permitted the measurement only of the carbon dioxide in the expired air. In the respiratory chamber of Pettenkofer and Voit, air entered freely and was drawn through a gas meter which measured its volume. The rate of ventilation was approximately 500,000 liters per

day. Samples of the incoming and outgoing air were collected at intervals and their content of CO₂ and H₂O determined. By determining the difference between the CO₂- and H₂O-content of the incoming and outgoing air and correcting for the total amount of air used up as measured by the gas meters, one could determine the amounts due to the respiratory processes of the living individual. A typical experiment gave the following data [6]:

Weight at start of experiment	71.090 kg	Weight at end of experiment	70.160 kg
Drinking water	1.0548 kg	CO ₂ given off	0.7383 kg
	<u>72.1448 kg</u>	H ₂ O lost	0.8289 kg
		Urine	1.1975 kg
			<u>72.9247 kg</u>

Difference between 72.9247 and 72.1448 = 0.7799 = oxygen utilized

Analysis of urine: 11.33 grams N
 5.81 grams C

C content of expired air, calculated from CO₂: 201.30 grams C

Total excretion of C: 5.81 + 201.30 = 207.11 grams C

(In starvation, the carbon content of the stool may be ignored.)

Using the nitrogen excretion as a starting point, Pettenkofer and Voit used such experimental data to calculate the amounts of various foodstuffs metabolized: Each gram of nitrogen excreted represents 6.25 grams of catabolized protein. Since 11.33 grams of nitrogen were excreted in the experiment above, the experimental subject catabolized 11.33×6.25 = 70.81 grams of body protein during the period of starvation. Analysis shows that the molecular structure of meat protein is such that 1 gram of nitrogen corresponds to 3.28 grams of carbon. In the experiment, 11.33 grams N were excreted, representing 11.33×3.28 = 37.16 grams C derived from catabolized protein. Subtracting 37.16 from the total amount of C excreted (207.11 grams) gives 169.95 grams C which cannot come from metabolized protein. This carbon is derived from the oxidation of carbohydrates and fats. Voit assumed that in the first stages of starvation there is no oxidation of carbohydrates and the stores of glycogen remain intact. The 169.95 grams C must therefore come from oxidation of fat and, since fat contains 76.52% carbon, the 169.95 grams of carbon correspond to 222.1 grams of fat.

During the experimental period of starvation, the subject therefore oxidized 70.81 grams of protein and 222.1 grams of fat. Confirmation of Voit and Pettenkofer's calculations was later made by determining the amount of oxygen required for the in vitro oxidation of proteins, carbohydrates, and fats:

100 grams protein	require	133.43 grams oxygen
100 grams carbohydrate	require	118.5 grams oxygen
100 grams fat	require	288.5 grams oxygen.

Thus, Voit's fasting subject should have required 735.24 grams of oxygen, as compared with the actual experimental value of 770.9 grams. If the 169.95 grams C did not come, as Voit supposed, from fat, but rather from carbohydrate, only 452.7 grams O₂ would have been necessary. The calculation of the respiratory quotient from Voit's published data (0.67) also confirmed his conclusion. However, there is an error in these studies, for it has been shown that during starvation there is first oxidation of a small amount of free, readily-available glycogen, amounting to approximately 150 grams (Benedict). Nonetheless, the value of Voit's basic contribution, which first helped to point the direction toward a detailed understanding of metabolic processes, remains undiminished.

After Voit and Pettenkofer had shown that one could determine the metabolites oxidized by calculations of the excretion of nitrogen and carbon dioxide and of the utilization of oxygen, they extended their studies to problems of nutrition. They investigated the degree to which fat, protein, and carbohydrate intake influence the oxidation or storage of foodstuffs, and the effects of physical activity, rest, and temperature on the processes of oxidation. They found that only protein and fat are broken down during starvation; that during physical activity both carbohydrate and fat are catabolized; and that during sleep, with absolute rest, the oxidation of fat is virtually at a standstill. They further showed that a diet composed exclusively of protein is able to maintain the amounts of protein and fat in the body; that when the diet is rich in protein, any excess dietary fat is stored; and that muscular activity does not increase the turnover of protein.

Lavoisier had already demonstrated in 1789 that an excess of oxygen does not increase the rate of metabolism. Voit now showed that the energy requirement of the body and the amount of food ingested determine the oxidative processes and the corresponding uptake of oxygen by the body. From this fact it also follows that respiration is a function of the processes of metabolism while, conversely, under physiological conditions respiration has no influence on metabolism.

The study of metabolism progressed rapidly in the following years, especially in the German and American schools. Important developments are linked with the names of the physiologists Rubner, Zuntz, Lusk, Benedict, DuBois, and the clinicians Friedrich von Müller, Magnus-Levy, Grafe, Means, and Boothby. From the middle of the 19th century, beginning with Liebig, the emphasis was also on the chemical nature of foodstuffs and metabolic products. Chevreul studied the chemistry of fats; Albrecht Kossel and Emil Fischer investigated the chemistry of proteins; and Emil Fischer also determined the chemical structures of various carbohydrates. With the discovery of the first cell-free enzyme system by Buchner, the era of enzyme chemistry began and rapidly led to the isolation of substances involved in intermediate metabolism (Harden, Embden, Neuberg). The road was now clear to an explanation of the processes of oxidation within the body, which Heinrich Wieland had first correctly conceived of as dehydrogenation. The hydrogen ions and electrons after dehydrogenation react, chiefly after a chain of complicated reactions, with oxygen (Otto Warburg, von Euler, Keilin). The catabolism of the foodstuffs proceeds rapidly, usually by way of high-energy acyl compounds, into the universal citric acid cycle of Krebs and Knoop and Martius, in which the largest part of carbon dioxide turnover takes place. The basic studies on the chemistry of energy metabolism (i. e., the production of chemical energy rather than heat as a result of oxidation) were made by Otto Meyerhof and A. V. Hill. Meyerhof developed the concept that certain coenzymes transfer energy, and Meyerhof's students and followers evolved the concepts of high-energy phosphate and of substrate phosphorylation and the respiratory chain (Lohmann, Lipmann, Kalckar, Lehninger, Lynen).

All these advances in the theories of energy metabolism would have been impossible without insight into certain basic facts. Among these are the demonstration of the protein nature of enzymes and Sumner's crystallization of urease, which gave the impetus for the isolation and crystallization of many other enzymes. Of comparable importance were Otto Warburg's improvement of the Barcroft apparatus and his introduction of optical tests for the determination of enzymes and coenzymes. Fiske and Subba Row developed a rapid method of measuring phosphate. Most recently, the introduction of isotopes into the study of metabolism has opened new possibilities for

metabolic research and has already led to important knowledge of the dynamic state of body constituents (Schoenheimer).

In 1929, Thannhauser closed the historical introduction to his book with the comment, "We stand here at the beginning of our knowledge of the most important problems of physiology and pathology. Humoral pathology, once a purely philosophical hypothesis, is about to become fact." Thirty years have now passed. The science of metabolism has been erected on the basic knowledge of the ancients. Each year has brought new discoveries, solved problems, and proposed new problems. Certainly there is no longer any doubt of the correctness of the concept of humoral pathology. There are already schools which seek to explain physiology and pathology on the basis of biochemical reactions alone. The future will determine if such concepts are true. At the moment, it is more reasonable to consider changes in form and changes of metabolism as equally important processes within the body. Although this book deals only with metabolism, it must always be recalled that life, insofar as we can describe it today, consists of a physical body through which there is a ceaseless passage of metabolic substances.

Physiology of General Metabolism

Introduction

The basic principles of thermodynamics hold as well for the metabolic processes of living organisms as for inanimate objects. Matter and energy are identical, so that changes in matter (metabolism) correspond to changes in energy.

The first law of thermodynamics is the law of conservation of energy, and states that the sum of all the forms of energy within an isolated system remains constant, independent of changes which may occur in the various forms of energy.

The second law is empirical. It states that, whenever there is a difference between energy potentials, there is a tendency for such changes in energy to occur that the potential difference will vanish, provided an energy exchange is physically possible. In this sense, all events occurring in nature are irreversible, and completely reversible reactions cannot occur. The entropy of a system is that part of the energy which cannot be re-utilized within this system. The source of energy of the animal body is food, and within food, energy is present in the form of chemical bonds. This energy is released by the processes of metabolism. After numerous transformations, the energy is finally dissipated as heat. Heat can thus be considered as the entropy of animal life. External work reduces the energy content of the body.

General Metabolism and Energy Turnover

Metabolism is the term employed to designate the sum of all chemical reactions which take place in the living individual. Two different functions, which cannot always be separated from each other, are part of the processes of metabolism: the preparation of the building-stones of the body from the diet, including the synthesis of various substances which go to make up the body tissues ("building-stone metabolism"); and the transformation of the type of energy present in food into forms of energy which can be utilized by the body tissues ("energy metabolism"). In the last analysis, all forms of energy developed by the processes of animal metabolism are derived from food, which is the only source of such energy. If the type and caloric

value of a given diet are known, it is possible to determine the amount of food oxidized and of energy liberated by determining the oxygen uptake and the excretion of carbon dioxide and certain nitrogenous compounds (the end products of metabolism). The validity of this type of calculation was shown by Lavoisier's experiments which correlated the production of carbon dioxide and the production of heat.

Following Lavoisier's fundamental experiments, research in metabolism progressed as the science of physics developed. In the first half of the 19th century, advances in metabolism included the mechanical equivalent of heat (Joule), the formulation of the concepts of energy maintenance (Julius Robert Mayer), and the indestructibility of matter and energy (Helmholtz). All forms of energy could now be calculated as heat and thus compared with each other, and it became certain that the same chemical processes gave the same energies whether *in vitro* or *in vivo*.

Despretz (1792-1863) and Dulong (1785-1838) nonetheless maintained, from animal experiments, that some of the heat produced must come from sources other than the diet. However, their method of calculating the caloric value of foodstuffs from their carbon and hydrogen content was erroneous. In contrast, Bidder and Schmidt [4] showed that the caloric value of the foodstuff itself must be used in the calculations. (These authors also recognized the necessity of correcting for the caloric value of excreted urea.) The point was conclusively settled by Voit and his school [7]. Later studies by Rubner, Atwater and Rosa, Benedict, and Lusk and DuBois showed that the amounts of oxygen utilized, carbon dioxide produced, and energy developed are *exactly* identical, whether the oxidation of foodstuffs occurs in the human body or in the calorimeter. When nitrogen-containing compounds (proteins) are burned in the body, the end products are urea and ammonia; in the calorimeter, however, combustion of proteins gives rise to nitrate, resulting in a higher caloric yield. If one adds the caloric value of the end products of animal protein metabolism (urea and ammonia) to that generated in metabolism, the sum of the calories is the same as in the complete combustion of the proteins in the calorimeter.

In the metabolism of the normal, full-grown adult organism, anabolic processes are in equilibrium with catabolic, and *all* chemical energy taken in is transformed into either heat or work. If there is no work – as, for example, under conditions of rest – energy can be given off only as heat. In man, who is homeothermic, the heat given off equals that produced. This heat comes from two sources: in part, it consists of heat lost during chemical reactions which are involved in liberating energy for the processes of life, and in part it is the final form of all forms of energy produced in the body. Energy which is not converted to heat within the body is not lost, need not be replaced, and thus does not enter into the energy balance of the body. Calorimetry of such a resting individual should therefore give values for the energy turnover which correspond to that calculated from gas and nitrogen metabolism, and this is actually the case. The same is true for an individual who performs work provided that the work produces heat and the heat is included in the calculations. The situation is different for the body which performs in the calorimeter work which produces potential energy (such as the lifting of weights onto a table). Insofar as the resulting potential energy can be measured, the following equation holds:

$$\begin{aligned} &\text{Heat formed (calorimetrically)} + \text{Final potential energy} \\ &= \text{Chemical energy turned over (gas analysis)}. \end{aligned}$$

In an organism which is still growing, a portion of the energy formed by the oxidation of foodstuffs is utilized for syntheses and thus enters into chemical bonds.

The oxidation of foodstuffs has taken place, as the end products demonstrate, but in part it has led to the storage of chemical forms of energy within the body. In such cases, calorimetry and gas analysis give different results. Under special conditions (for example, in the conversion of carbohydrate to fat) the divergence can be corrected, but in general it can only be guessed at.

Calculation of Heat of Oxidation (Caloric Value)

The large calorie (kilogram-calorie, Kcal, or, simply, Calorie) is the unit of measure. In any discussion of energy balance, it is first necessary to know the caloric value of the foodstuffs. These values are determined by direct calorimetry in the bomb calorimeter. Rubner [8], among others, has used this device to calculate the caloric values of common foodstuffs (Table 1). The caloric value for protein is an average, after correction for the energy content of the nitrogenous compounds, calculated by Rubner on the assumption that the diet of man contains animal and plant proteins in a ratio of 3:2. The caloric value of animal protein is 4.23 Calories per gram; that of vegetable protein, 3.96 Calories per gram.

One gram of protein and one gram of starch release the same amount of heat, 4.1 Calories. For the production of this amount of heat, only $\frac{4.1}{9.3} = 0.44$ grams of fat are necessary. Rubner used the term "isodynamic" to describe the amounts of different foodstuffs which are equivalent calorically. His "isodynamic law" described the interchangeability of foodstuffs with regard to energy. This is merely a special case of the general law of conservation of energy. Its value is somewhat limited because dietary foods often cannot be interchanged at will in isodynamic amounts (see, for example, the section on protein minimum).

Table 1. Metabolic and Energy Changes in the Oxidation of Foodstuffs

Turnover in oxidation of 1 gram of foodstuff	Oxygen uptake c. c.	CO ₂ formed c. c.	R. Q.	Calories produced according to			Caloric equivalent of	
				Rubner [8]	Loewy [9]	Zuntz [1]	1 liter O ₂	1 liter CO ₂
Protein	966.3	773.9	0.801	4.01	4.316	4.3	4.485	5.579
Urinary N	5939.0	4757.0	0.801	25.63	26.54	—	4.485	5.579
Fat	2019.3	1427.3	0.707	9.3	9.461	9.4	4.686	6.629
Starch	828.8	828.8	1.000	4.1	4.182	4.2	5.047	5.047
Fasting (14 hours after last meal)	—	—	0.82	—	—	—	4.8	—

Table 2. Physical Properties of Gases. From DuBois [12]

Gas	Molecular weight	Coefficient of solubility in water at 0° C	Weight of 1 liter (grams)	Volume of 1 gram (liters)
Oxygen	32,000	0.049	1.4292	0.6997
Carbon dioxide	44,005	1.71	1.963	0.5094
Nitrogen	28,016	0.023	1.2542	0.7973
Water vapor	18,016			

The equation of oxidation for starch is as follows:



After division by n and insertion of the molecular weights, this becomes



These equations are instructive in various ways. First, they allow us to calculate the amount of oxygen necessary for the oxidation of 1 gram of starch:

$$\frac{192 \text{ grams}}{162} = 1.185 \text{ grams}.$$

This can be more conveniently expressed as volumes (Table 2):

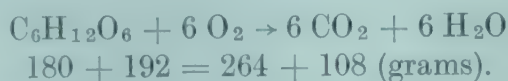
$$1.185 \times 0.6997 = 0.829 \text{ liters}.$$

Thus, the oxidation of starch gives, per liter of oxygen (using Loewy's data)

$$4.18 \times \frac{1}{0.829} = 5.05 \text{ Calories}.$$

(Amounts of gas are usually expressed as the volume at 760 mm Hg, 0° C, dry. Volumetric measurement permits the direct comparison of oxygen uptake and carbon dioxide output for, according to Avogadro's law, gases at the same pressure, temperature, and volume have the same number of molecules and are therefore of the same molarity.) The energy value of 1 liter of carbon dioxide liberated in the oxidation of starch must be the same, for the amounts of oxygen utilized and of carbon dioxide formed are isomolar and the volumes are thus the same.

The same calculation for glucose gives the following results:



The oxygen necessary for oxidation of 1 gram of glucose is

$$\frac{192}{180} = 1.067 \text{ grams, or}$$

$$1.067 \times 0.6997 = 0.747 \text{ liters}.$$

The caloric value of 1 gram of glucose is 3.75 Calories. A liter of oxygen therefore produces

$$\frac{3.75}{0.747} = 5.02 \text{ Calories}.$$

Thus, although the caloric value of carbohydrate differs with different types of carbohydrate, 1 liter of oxygen always produces the same number of calories in carbohydrate oxidation. It is often not realized, however, that the oxidation of 100 grams of starch results in the formation of

$$90 \times \frac{100}{162} = 55.5 \text{ grams of water!}$$

The calculation of the oxidation of fat can be made from a hypothetical triglyceride, such as tristearine. Because of the unsaturated fatty acids present in the natural fats, such calculations give a low R. Q. It is better to analyze the fat to be oxidized and to calculate the R. Q. from the results of the analysis. Lehmann, Müller, Munk, Senator and Zuntz [11] performed classical experiments in this regard. They found a respi-

ratory quotient of 0.709 at a caloric equivalent of 4.69 Calories per liter of oxygen, and 6.63 Calories per liter of carbon dioxide. More recent analyses have left this value practically unchanged (Table 3).

Table 3. Values for the Oxidation of Fat [13, 14, 15]

Source	R. Q.	Caloric equivalent of 1 liter O ₂	Calories per gram of fat
[13] Human, abdominal wall	0.711	4.75	9.51
Human, liver	0.719	4.65	9.11
Human, muscle	0.717	4.82	9.23
Human, obese abdominal wall	0.712	4.79	9.45
[14] Butter	0.720		
[15] Horse	0.710		9.46
Ox	0.711		9.51
Sheep	0.721		9.45
Hog	0.719		9.51
Dog	0.723		9.49

It is interesting to study the formation of water in this process: 100 grams of the fats studied by the authors gave 107 grams of water. The body obtains large amounts of water from the diet in this way, and this fact must be taken into account in any studies of water balance.

The calculation for protein is somewhat complicated [9]:

100 grams meat protein 52.38 g C 7.27 g H 22.68 g O 16.65 g N 1.02 g S

The corresponding values excreted in the urine are

9.406 C 2.663 H 14.099 O 16.28 N 1.02 S

The corresponding values excreted in the feces are

1.471 C 0.212 H 0.889 O 0.37 N

The amounts remaining for the calculation of the gas metabolism are

41.50 g C 4.40 g H 7.69 g O

For the oxidation of C and H, the amount of O necessary is

$\frac{41.5 \times 32}{12} + \frac{4.40 \times 16}{2} = 110.67 + 35.2 = 145.87 \text{ grams.}$

Of the total, 7.69 grams of oxygen are available from the protein, and 138.18 grams must be supplied.

The caloric values of the gases for the oxidation of 1 gram of protein can then be calculated for the oxygen taken up:

$1.382 \times 0.6997 = 0.9668 \text{ liters O}_2$
 $\frac{4.316}{0.967} = 4.463 \text{ Calories/liter.}$

For the carbon dioxide excreted, the calculation is

$\frac{41.50 + 110.67}{100} \times 0.5089 = 1.522 \times 0.5089 = 0.7745 \text{ liters CO}_2$
 $\frac{4.316}{0.7745} = 5.572 \text{ Calories/liter.}$

In the case of the proteins, the energy metabolism can also be calculated from the amount of urinary nitrogen, since the nitrogen in the urine comes only from the protein. The catabolism of 100 grams of animal protein results in the production of 16.28 grams of nitrogen in the urine, which corresponds to 96.68 liters of oxygen taken in, 77.45 liters of carbon dioxide given off, or 431.6 Calories. Division of these figures by 16.28 gives the corresponding values for 1 gram of urinary nitrogen (Table 1).

Loewy's data have not been recalculated recently. As in the case of the fats, the factors for the caloric equivalent of oxygen and of urinary nitrogen must be changed depending on the origin of the protein. Table 4 lists the data for certain special proteins.

Table 4. Values for the Oxidation of Protein [16, 17]

Source	R. Q.	Caloric equivalent of 1 liter O ₂	Calories per gram of protein (or g urinary N)	Oxygen consumption (liters) per gram of urinary N
Gelatin [16]	0.833	4.694	3.418	4.70
Urea N after gelatin			(22.08)	
Casein [17]	0.821	4.586	—	6.67
Urea N after casein			(30.59)	
Meat protein (Loewy)	0.801	4.463	4.316	5.94
Urea N after meat protein			(26.54)	

Respiratory Quotient and Calculation of Total Metabolism

The respiratory quotient (R. Q.) is the molecular ratio between the carbon dioxide formed and the oxygen taken up; i. e., $\frac{\text{CO}_2}{\text{O}_2}$. For carbohydrates, the R. Q. is 1; for fats, it is usually given as 0.707, but is probably somewhat higher; and for protein, it is given by Loewy as 0.801. Since the R. Q. is different for different foods, its numerical value gives an idea of the type of foodstuffs being utilized. In addition, the ratio between two known foodstuffs in a mixture can be calculated from the R. Q. For the calculation of the total metabolism, however, the amount of protein catabolism is calculated from the urinary nitrogen. After subtracting from the urinary nitrogen that portion due to the corresponding amounts of carbon dioxide and oxygen, the remaining R. Q. is that for fat and carbohydrate together, and the percentage of each in metabolism can thus be calculated. The following example illustrates the calculation, using the values of Table 1 (DuBois, 12):

Urinary nitrogen	0.395 grams per hour
CO ₂ excretion	11.058 liters per hour
O ₂ uptake	13.882 liters per hour
Calories due to protein	0.3895 × 26.54 = 10.34 Cal.
CO ₂ of protein	0.3895 × 4.757 = 1.855 liters
O ₂ of protein	0.3895 × 5.939 = 2.316 liters

The R. Q. after subtraction of protein catabolism is given by

$$\frac{11.058 - 1.855}{13.882 - 2.316} = \frac{9.203}{11.566} = 0.795.$$

If one uses the figure 0.707 as the R. Q. of fat, it is easy to calculate that, at an R. Q. of 0.795, 70% of the oxygen is used for the oxidation of fat.

$$\frac{(\text{CO}_2 \text{ from fat}) + (\text{CO}_2 \text{ from carbohydrate})}{(\text{O}_2 \text{ for oxidation of fat}) + (\text{O}_2 \text{ for oxidation of carbohydrate})} = 0.795$$
$$\frac{(\text{CO}_2 \text{ from fat})}{(\text{O}_2 \text{ for fat})} = 0.707; \text{ hence } (\text{CO}_2 \text{ from fat}) = 0.707 (\text{O}_2 \text{ for fat})$$
$$\frac{(\text{CO}_2 \text{ from carbohydrate})}{(\text{O}_2 \text{ for carbohydrate})} = 1.0;$$
$$\text{hence } (\text{CO}_2 \text{ from carbohydrate}) = (\text{O}_2 \text{ for carbohydrate})$$

Hence, by substituting,

$$\frac{0.707 (\text{O}_2 \text{ for fat}) + (\text{O}_2 \text{ for carbohydrate})}{(\text{O}_2 \text{ for fat}) + (\text{O}_2 \text{ for carbohydrate})} = 0.795$$

The equation can now be solved for the proportion of O₂ used for the oxidation of fat and carbohydrate.

The last phase of the calculation has already been made in the Zuntz-Schumburg Table [10], which also gives the caloric equivalent per liter of oxygen for each R. Q. According to Lusk's modification of this table [18], this caloric equivalent at an R. Q. of 0.795 is 4.796 Calories, and the production of calories from fat and carbohydrate can be determined as follows:

$$4.796 \text{ Calories} \times 11.566 = 55.47 \text{ Calories.}$$

The total calories produced are then given by

$$55.47 \text{ Calories} + 10.34 \text{ Calories} = 65.81 \text{ Calories per hour.}$$

The results obtained by these calculations are in good agreement with those obtained by direct calorimetry.

If the diet of a subject under study is very different from the normal diet, the Zuntz-Schumburg table usually fails. Thus, for example, erroneous results were obtained in two subjects whose diet was predominantly meat. According to the Zuntz-Schumburg table, these subjects oxidized 34 grams of carbohydrate more than they actually took in their diet [19]. This discrepancy is due to the very low R. Q. of fat used by Zuntz (0.707). Cathcart and Cuthbertson [13] have given a modern modification of the table based on higher R. Q. values for fat (Table 5). Actually such a change has little effect on the calculations for a normal diet, as can be shown by recalculating the above problem using the newer figures. The new results show that 72.4% of the calories come from oxidation of fat, and the total metabolism measures 66.09 Calories per hour. The difference is only 0.4%.

Table 5. Zuntz-Schumburg Table, Modified by Cathcart [13]

R. Q.	Percentage of oxygen consumption		Percentage of heat formation		Caloric equivalent of 1 liter O ₂
	Carbohydrate	Fat	Carbohydrate	Fat	
0.718	0.0	100.0	0.0	100.0	4.735
0.75	11.4	88.7	12.0	88.0	4.770
0.80	29.1	70.9	30.4	69.6	4.826
0.85	46.8	53.2	48.4	51.6	4.881
0.90	65.5	35.5	66.0	34.0	4.936
0.95	82.3	17.7	83.2	16.8	4.992
1.00	100.0	0.0	100.0	0.0	5.047

These calculations can be simplified for clinical use. Magnus-Levy [20] found that the fasting individual (i. e., last meal 12 to 14 hours before study) obtains 12 to 18% of his calories from protein. This fact can be combined with the data of the Zuntz-Schumburg table to facilitate calculation of the metabolic rate. If only the basal metabolic rate is required, the error by this simplified method is less than 1%. Tables which do not take protein catabolism into account give, even under basal conditions, values which are 1 to 1.5% too high, and should not be used. In balance experiments in which the uptake of food on the one hand and the formation of heat on the other hand are followed (gasometric technic), the literature is often in error because Rubner's figures are used for the caloric content of foodstuffs, while gas-metabolism studies use the data of Loewy. As seen in Table 1, the caloric values of the individual foodstuffs are given differently by these two authors.

When the R. Q. is known, the energy turnover can be calculated from the uptake of oxygen or the output of carbon dioxide. Technically, it is simpler to measure carbon dioxide exactly, but the determination of the oxygen uptake has turned out to be more practical for two reasons. Firstly, the amount of carbon dioxide given off depends on the depth of breathing and hyperventilation can give high CO_2 values. Secondly (Table 1), the caloric equivalent of oxygen is much less dependent on the type of foodstuff catabolized (and therefore on the R. Q.) than is that of carbon dioxide. In calculations of energy turnover based on the output of CO_2 , therefore, a small error in gas analysis would influence the results much more than in calculations based on the uptake of oxygen. (The caloric equivalent of oxygen is not sufficiently constant to give the energy turnover without a knowledge of the R. Q. except under basal conditions, when the R. Q. is comparatively constant and lies between 0.785 and 0.800.)

The proportion of heat lost by evaporation remains quite constant under certain conditions. This fact allows the calculation of the basal metabolism from the amount of insensible perspiration [21]. Johnston and Newburgh [22] devised a method of determining the total metabolism from the amount of water given off through the skin and the lungs. In careful hands, this method gives valuable data. However, the ratio between water output and heat production does not hold for sick individuals [23, 24] or for persons who sweat considerably [25, 26], and the ratio also changes when there is a marked increase in the metabolic rate [27].

Convertibility of Foodstuffs

Observations on the fattening of animals have demonstrated that it is possible to cause deposition of fat by feeding only carbohydrates. The ability of the body to store glycogen is small, so that glycogen is not an important reservoir of energy. The body rapidly converts a portion of absorbed carbohydrate into fat. From the standpoint of metabolism, body fat has a rapid turnover. Labelled fatty acids are rapidly taken up and given off by the body fat. The observation that animals can take in all the energy necessary for a day in one meal is also in favor of the participation of fat in short-term storage of energy -- the stores of glycogen alone are so small that they cannot store energy for an entire day.

The conversion of glucose into fat can be summarized in the following type of equation for a mixture of palmitic and stearic glycerides:



Oxygen is formed in the course of this reaction, so that the R. Q. of the conversion process has a negative value. Negative values of the R. Q., however, never occur in

practice. In addition, the R. Q. is rarely higher than 1.2. If the fat which is formed in the equation above is oxidized, 80 molecules of O_2 are used up and 54 molecules of CO_2 are formed. Thus, in addition to the $8 + 3 = 11$ molecules of O_2 which are already present, an additional 69 molecules of O_2 must be supplied. Since the total CO_2 formed (on the right side of the equation) is $54 + 15 = 69$ molecules, the R. Q. of synthesis of fat from carbohydrate followed by oxidation of the fat measures 1.0. This result is as expected, but it must be understood that when the R. Q. is smaller than or equal to 1, the synthesis of fat proceeds more slowly than its oxidation, so that the total catabolism can be correctly calculated from the gas metabolism, even when fats are formed from carbohydrates in intermediary metabolism.

The R. Q. described above is the total R. Q. *after* the subtraction of the protein metabolism. Thus, in the presence of simultaneous oxidation of protein, a total R. Q. of 1.0 is an indication of synthesis of fat, for the subtraction of the amount of gas due to the protein metabolism raises the basal R. Q. above 1. If the R. Q. is greater than 1, energy metabolism cannot be calculated either from direct calorimetry or from data obtained by gas analyses. In calorimetry the energy liberated from carbohydrate cannot be measured since it is partly stored as fat. Gas analyses do not permit conclusions because calculations based on such results actually are based on the complete combustion of foodstuffs.

The conversion of fat – or, more exactly, of fatty acids – into carbohydrates was long a subject of bitter debate. Modern studies of intermediary metabolism by means of isotopes have shown beyond doubt that fat is converted to carbohydrate. This fact is no longer surprising, for fats, like carbohydrates, are ultimately catabolized in the citric acid cycle (i. e., fats and carbohydrates have common pathways of catabolism). However, the conversion of fat into carbohydrate plays only a very small role in metabolic balance. The ability to store glycogen is limited, while the fat depots can be expanded. In practice, there is no proof for any quantitatively important conversion of fat into carbohydrate. Such a conversion would cause an R. Q. less than 0.707, which has never been observed with careful technics. In addition, animals with total diabetes do not excrete more glucose than that which corresponds to their intake of protein and carbohydrate.

Since animals fed almost exclusively with protein can be maintained in metabolic equilibrium, yet store fat or glycogen, the conversion of protein into carbohydrate and fat cannot be doubted. Such conversions are necessary since the body cannot store protein. The conversions can be easily understood from a study of the reactions of intermediary metabolism. The process of conversion in diabetes is of special interest. All the glucose found in the urine of a diabetic animal on a carbohydrate-free diet comes from protein, and the ratio of glucose to nitrogen (i. e., glucose to protein) – the D:N ratio of Minkowski [28] – is a measure of the ability of the body to convert protein into carbohydrate. After subtracting the carbohydrate present in the glycerol of fats, the upper limit of this ratio is 3. That is, $\frac{3}{6.25}$ or about half of the protein ingested can be converted into glucose.

Some Principles of Metabolic Balance

In man, the intake of food is generally periodic while the production of heat and the processes of synthesis are continuous. The constant rate of utilization of energy is occasionally interrupted by physical activity. In other words, the amount of energy

taken in is only rarely equal to the amount of energy spent. The calculation of a metabolic balance therefore depends on several prerequisites.

It is obvious that there is no "metabolism" unto itself, but that the word encompasses many individual metabolic processes. The phrase "general metabolism" is often used, however, for energy turnover. With regard to balance, it is the individual metabolic processes which are of importance as, for example, the metabolism of the nitrogen-containing compounds, the inorganic substances, the oxygen, and the total calories. In some cases - e. g., the minerals - such balances are simple, for the substances studied do not undergo alteration within the body. In other cases - e. g., nitrogen, oxygen - these balance studies become possible by virtue of the fact that only the outermost components of a chain of reactions are considered. In many cases, it is possible to perform partial balance studies, but in some cases even such studies are impossible. The introduction of labelling technics with radioactive isotopes has opened up vast new possibilities in the study of metabolic processes, although uncritical application of these methods may give serious errors.

The determination of the magnitude of metabolism under given circumstances becomes significant only when a steady state is maintained during the period of observation, or when the metabolic process under study is completely observed

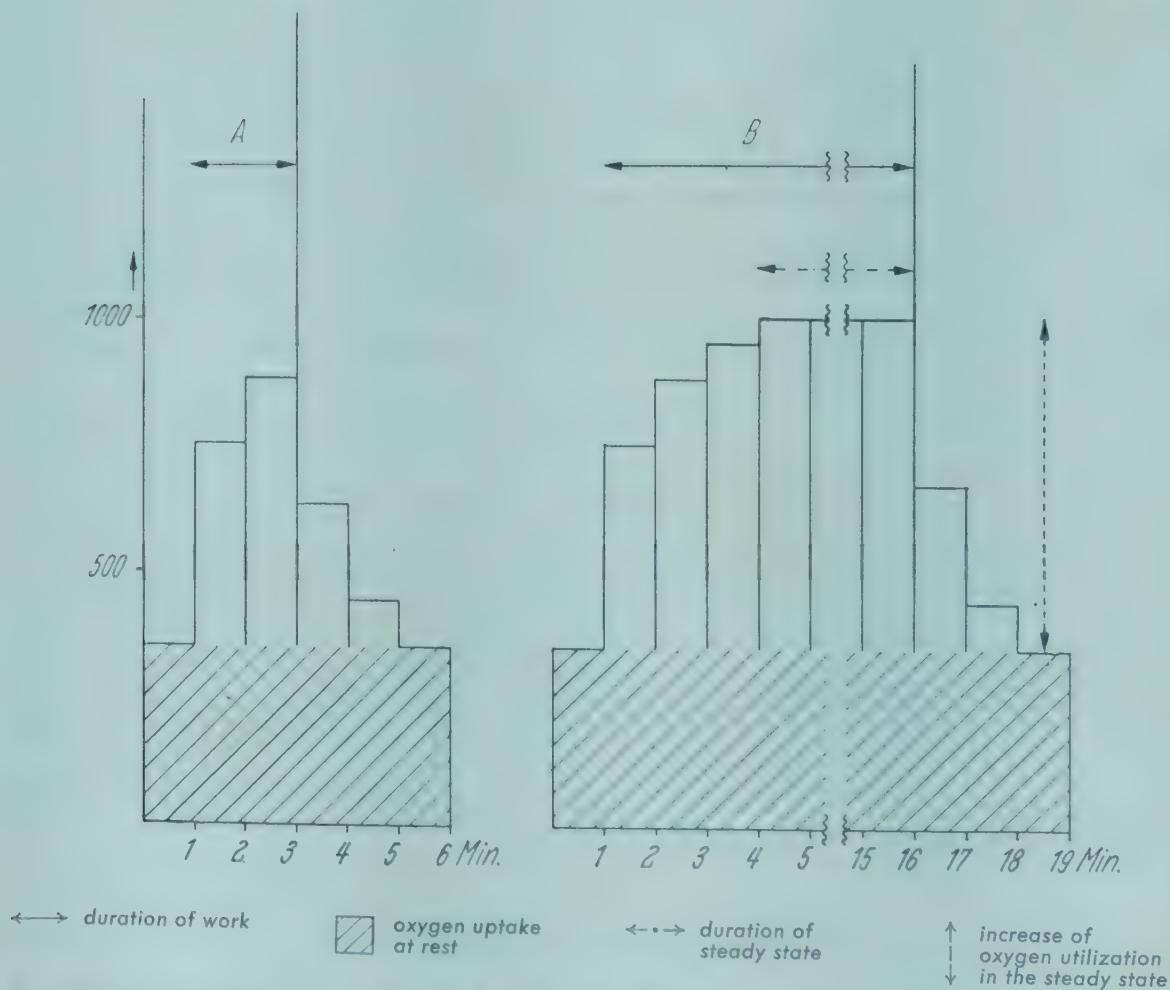


Fig. 1. Oxygen utilization in muscular effort of varying duration. - A: short-termed effort (2 minutes). - B: longer effort. After 3 minutes, a steady state is reached with respect to the oxygen uptake. - Abscissa: time in minutes. - Ordinate: oxygen uptake.

between two comparable states. A "steady state" is that in which the velocity of the observed reactions does not change with time – i. e., the velocity is constant. In the presence of a steady state, the results of measurement over a suitable time interval allow calculation of the entire process. The classic example is the basal metabolism. The foodstuffs of the body steadily decline, oxygen is steadily used up, and carbon dioxide, water, and urea are constantly formed. On the other hand the substances involved in intermediary metabolism – whether in glycolysis, the citric acid cycle, or the synthesis of urea – have concentrations which do not change as long as the steady state is maintained ("stationary concentrations"). Other examples include the synthesis of fat after carbohydrate feeding and the processes of oxidation during a prolonged but not exhausting period of activity. The steady state is bound to a certain magnitude of metabolic processes. If this is changed by the body, the steady state is replaced by an "unsteady state," which can again change to a steady state in time.

The quantitative description of a metabolic process in the steady state is comparatively simple. Frequently, however, metabolic processes occur in such a way that a steady state is never attained. In such cases, significant balances can only be established when the metabolism returns to its starting point (usually a steady state) after passage through various unsteady states. This can be seen, for example, in nitrogen balance. If we assume that the concentrations of the intermediate substances from digestion, absorption, and utilization are the same in a fasting individual on two successive days, then all the nitrogen which enters the body in the interval must be present on the second day in the end products – i. e., it must be excreted or stored. The comparison of uptake and excretion thus allows the development of a nitrogen balance. Given this balance, the nitrogen metabolism within the 24 hours of observation can be studied, but any inference concerning any specific period cannot be made. (In the case of nitrogen metabolism, this is obvious. In other cases, the temptation to make conclusions concerning short periods from the total balance is greater, but such conclusions can be made with validity only when a steady state is present throughout the entire balance period.)

Measurement of Energy Turnover by Means of the End Products of Metabolism

The calculations of protein catabolism have already shown (p. 11) that almost all of the nitrogen ingested in proteins appears in the urine, with a small percentage in the stool. The loss of nitrogen in the hair and the nails is very small and amounts to only 30 mg daily [29]. The loss through the skin is greater, and under resting conditions amounts to some 70 mg of N per day [30]. During light physical activity, this value rises rapidly, and during severe activity or marked sweating the excretion may reach levels as high as 220 mg per hour [30, 31, 32]. This comparatively large amount of nitrogen in the sweat is probably the result, however, of an increased catabolism of protein and there is no reduction of nitrogen excretion in the urine. The expiratory air, even in prolonged experiments, shows no ammonia [33, 34].

The nitrogen excreted in the sweat consists chiefly of urea and ammonia and can be included as urea nitrogen in the calculations of gas metabolism. The nitrogen of the stool, on the other hand, is derived chiefly from protein. Under physiological conditions, the stool protein does not come from protein in the ingested food but from intestinal secretions which have not been reabsorbed [35]. Albanese [36] isolated a protein in the stool of children which he called "fecanin" and which accounted for

some 22% of the nitrogen in the stool. Prausnitz [37] showed that the composition of the stool is constant under various dietary conditions. The increased amount of protein in the stool after ingestion of meat seems to be related to increased digestion and not to diminished absorption [38, 39]. The source of the individual stool proteins, however, is not known. In man, bacteria comprise not more than 11% of the dried stool.

Since the nitrogen of the stool is derived from protein which has not been oxidized, one may consider the nitrogen of the urine as the only nitrogenous *end* product of protein metabolism (with due consideration for the nitrogen of the sweat). Urinary non-protein nitrogen may thus be used in the calculations of gas metabolism (Table 4). (On the other hand, the calculation " $\text{urinary N} \times 6.25 = \text{protein ingested}$ " ignores the protein content of the stool.) If, instead of non-protein nitrogen, only urea nitrogen is used in the calculation, the urinary content of ammonium salts, uric acid, and amino acids is ignored. If the urine contains pathological amounts of protein, this must be handled like the stool protein; i. e., it must be included in the calculation of nitrogen balance and ignored in the calculation of energy metabolism or heat production. Corrections are necessary for the nitrogen content of drugs. If the amount of protein oxidized during a given period of study is to be determined, the subject must receive a standard diet for two days before the study. When the period of study is short, it is even more important to make observations at a considerable interval after the last meal.

Under normal conditions, the fat in the stool is due to intestinal secretions and not to diminished absorption of dietary fat. Hill and Bloor [41] have shown that the composition of stool fat is quite independent of the diet, and it has been shown by others that almost as much fat is excreted when the diet is rich in fat as when it is fat-free [42].

Roughly speaking, the excretion of protein and fat in the stool can be considered to be constant. Some authors therefore do not bother to calculate these excretions and instead use a correction factor in their studies. Rubner [8] gives the caloric value of one gram of organic matter in the stool as 6.06 to 6.40 Calories, and 6.2 can be taken as an average value. A practical method of labelling the stool formed in a given period of time is to give carmine or charcoal before and after the experimental period. Studies of protein- or fat-metabolism require careful and complete collection of all specimens.

Most errors which arise in measurements of carbon dioxide output are due to a lack of a steady state. Thus, for example, some nervous people hyperventilate during determination of the basal metabolism and the exaggerated breathing results in an increased output of carbon dioxide, giving a falsely high R. Q. The increased output of carbon dioxide may persist for a relatively long period of time, for the gas which passes from the plasma is constantly replaced by carbon dioxide from the cells, so that the partial pressure in the blood falls only slowly to alkalotic levels. The phenomenon known as "pumping out" is followed by compensatory hypopnea and the R. Q. falls. The period of hypopnea, however, may not take place until after the experimental period is over.

In addition to this disturbance of the steady state as regards the formation of carbon dioxide during hyperventilation, there are other disturbances which are related to the buffer function of bicarbonate. When increased amounts of acid are formed by metabolic processes, bicarbonate is converted to undissociated carbonic acid, which then becomes carbon dioxide. This buffering action of bicarbonate is equivalent to an elevation of the partial pressure of the carbon dioxide in the blood and results in an increase in the respiratory rate. This type of situation occurs when keto acids are formed during the oxidation of fat and in diabetes, and when large amounts of lactic

acid – as much as 3 grams per second – are formed during physical activity [43]. The resulting elevation of the R. Q. is, of course, not an expression of the true metabolic state, for the carbon dioxide excreted is not derived directly from oxidative reactions. The situation is reversed when, at the end of the period of physical activity, the lactic acid is oxidized or is reconverted to glycogen. The carbon dioxide produced by the processes of oxidation is not exhaled but is converted into bicarbonate during the restoration of the normal buffer state. Similar events often occur in less drastic situations. Thus, for example, the infusion of fructose rapidly causes the production of lactic acid or pyruvate, and therefore leads, as described above, to an elevation of the R. Q. This R. Q., again, is not an expression of any increase in carbohydrate oxidation: there is no such increase.

Rosenbaum [44] showed that the administration of bicarbonate causes no notable change in the excretion of carbon dioxide by the lungs. Cell membranes are permeable to carbonic acid and to carbon dioxide, but not to bicarbonate [45], so that the administered bicarbonate remains completely in the plasma and in the extracellular fluids until it is excreted in the urine. The administration of bicarbonate thus does not interfere with gas analysis experiments. (On the other hand, considerable amounts of bicarbonate can be excreted in the urine in alkalotic states.) The influence of ammonium chloride on gas metabolism is also small, for it is only in the extracellular fluids that the chemical reaction



is shifted to the right. However, a large portion of the carbon dioxide liberated enters the cells and can be taken up by the buffer systems within the cells. This fact places the acidosis due to ammonium chloride in direct opposition to the true metabolic acidoses in which the acids also are present within the cells and in which large amounts of carbon dioxide are exhaled. A number of other factors which influence the excretion of carbon dioxide are listed in Table 6 [86]. It is therefore necessary to determine the

Table 6. Factors Which Affect the Excretion of Carbon Dioxide

I. Increased loss of CO ₂ above the amount of oxidation
a) Hyperventilation
b) Formation of acids
i) Lactic acid in severe work, convulsions, asphyxia, administration of epinephrine or insulin
ii) Keto acids in diabetes or carbohydrate-poor diets
c) Retention of acids: nephritis, uremia, hepatic coma
II. Reduced loss of CO ₂ (CO ₂ -retention)
a) Hypoventilation
b) Catabolism of acid and utilization of bicarbonate to restore the normal buffer system
i) Lactic acid in the recovery phase of muscular effort
ii) Keto acids in insulin therapy, improvement of diabetes, or cessation of a carbohydrate-poor diet
c) Excretion of acid in the urine
d) Loss of acid in vomiting
e) Alkalosis
i) Utilization of CO ₂ as buffer
ii) Excretion of alkali as bicarbonate

plasma bicarbonate or carbon dioxide in doubtful cases before and after an experiment and, if the values are not identical, the experiment must be discarded.

The oxygen requirement in muscular work is also a good example of the basic considerations in the measurement of metabolism. When the period of work has ended, the elevated uptake of oxygen does not fall immediately to resting values, but remains elevated for a certain period of time (Figure 1). If the amount of oxygen required for

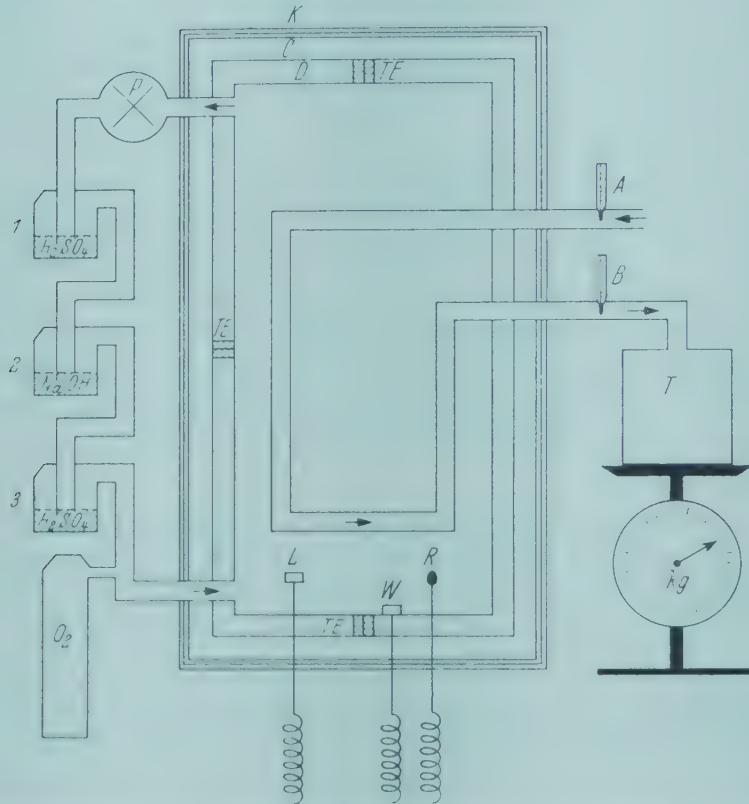


Fig. 2. Diagrammatic representation of a Respiratory Calorimeter. Modified from Lusk [18].

Ventilation system: The ventilator drives air through the absorption tubes, holding back water vapor (1), carbon dioxide (2), and water vapor from alkali (3). Oxygen is added as needed.

Gas analyses: Increase in (1) = water given off by the experimental subject

Increase in (2) + (3) = carbon dioxide given off

Decrease in oxygen = oxygen utilized

Calorimeter: A and B thermometers for incoming and outgoing water

T = tank for weighing the water passed through the calorimeter

R = rectal thermometer

W = thermometer for calorimeter wall

L = air thermometer

TE = thermal element uniting the copper walls of the calorimeter

C and D = air spaces

K = outer wall of cork

The thermal elements give the differences in temperature between the inner and outer copper walls, which can be equalized by cooling or warming, so that the calorimeter does not lose heat.

Calorimetry: (Mean difference between A and B) \times (liters of water) + (grams of water in [I]) \times (0.586*) + (change in the temperature of the wall of the calorimeter) \times (heat equivalent of the calorimeter**) + (change of body temperature) \times (heat equivalent of the body***) = total calorie formation****.

* Heat of evaporation of 1 gram of water at 20° C.

** Determined experimentally

*** 0.83

**** Omitting the last term on the left side of the equation gives the total calories lost.

the work is to be determined, the total oxygen uptake must be measured from the beginning of the work until the oxygen uptake originally present is again reached. The difference between the total oxygen uptake during and after work and the amount of oxygen consumed in an identical period of time during rest is equal to the oxygen requirement of the work (measurement in balance between two steady states). If a non-exhausting work can be maintained uniformly for a period of time long enough to reach a steady state, the increased uptake of oxygen in each time interval equals the increased utilization of oxygen. The calculation of the oxygen utilization of a uniformly continuous work can then be made from a knowledge of the results of the analysis during a given interval of time and the duration of the work. When the utilization of oxygen exceeds 2 liters per minute, a steady state can no longer be attained even in healthy individuals, and rapidly progressive exhaustion is characteristic of this state. At the borderline between exhausting work and work in a true steady state, the uptake of oxygen can be constant while the oxygen debt increases. In such cases the uptake of oxygen in a given interval with an apparently steady state is not representative of the actual utilization of oxygen.

Measurement of Total Metabolism

Lavoisier long ago described the two most important methods of measuring the production and output of heat during metabolism: direct calorimetry and gas analysis.

Exact methods of direct calorimetry were developed by Rubner, whose apparatus was built for experiments on dogs. Atwater and Rosa then constructed the first calorimeter suitable for studies on man [47]. F. G. Benedict introduced a closed system of respiration into this apparatus [48], and numerous studies with this calorimeter were made by Benedict, Lusk, and DuBois. The apparatus is pictured in Figure 2 and Lusk [49] has described the method in detail.

The pioneers of gasometric analysis (gas calorimetry) were Regnault and Reiset (1849), who were the first to measure the amount of carbon dioxide exhaled. Bidder and Schmidt developed an exact technic and Voit and his students improved the method. Gasometric calorimetry can be performed either in an open system (Zuntz and Geppert, Tissot) or in a closed system (F. G. Benedict).

In the older, open system, the person under study breathes in gas from a reservoir or breathes air, and the gas exhaled is collected in special containers, usually spirometers. The uptake of oxygen and the production of carbon dioxide can be calculated from the composition of the gases breathed in and the analysis of the

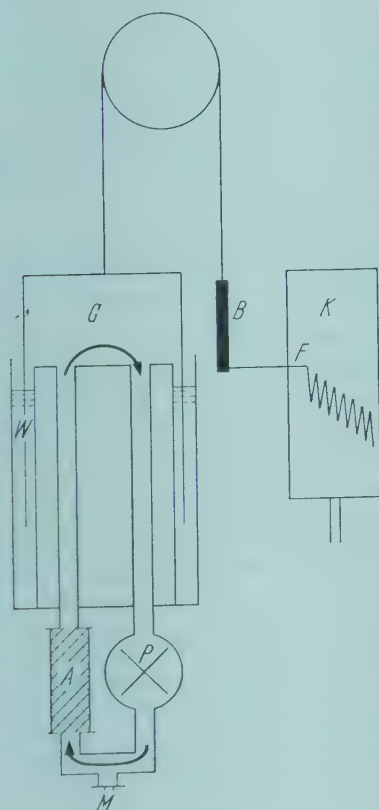


Fig. 3. Diagrammatic representation of a spirometer (Benedict-Roth).

The bell-jar G is filled with oxygen. The pump P causes gas to circulate in the direction of the arrow. The subject breathes in and out by means of a mouthpiece M. CO_2 of the expired air is absorbed by alkali in A. W is a water seal. The counterweight B balances the bell-jar and writes by means of the pen F on the kymograph K. The reduction of volume corresponds to the oxygen uptake of the subject.

exhaled air. Tissot's principle is basic for gas calorimetry [50], for errors and inexact analyses will be manifested in the results of the calculations [51]. The Douglas bag is a portable container in which the exhaled air is readily collected even when the subject moves about [52].

The principle of the closed system [53] is the same as that of the calorimeter. Benedict's first apparatus measured both the utilization of oxygen and the production of carbon dioxide. Later, the measurement of carbon dioxide was discarded [54]. Roth introduced valves to maintain the circulation of the gases [55] and Krogh independently devised a similar apparatus [56]. Figure 3 gives the principle of this method. Knipping again made possible the measurement of carbon dioxide in the Benedict-Roth apparatus by using a spirometer in which the carbon dioxide is absorbed by 47% potassium hydroxide, from which it is then liberated by means of dilute sulfuric acid and measured volumetrically [57]. However, this technic is difficult and in practice only the oxygen utilization is measured in closed system apparatus. This method is ideal for the determination of the basal metabolism for the R. Q. remains constant. (Under other conditions, if the R. Q. is not constant, errors up to 3.6% can occur.) The closed system technic is simple, but gas leaks and improper calibration of the thermometer, kymograph, etc., can cause serious errors which may remain undetected unless controls are constantly performed. Duplicate determinations should agree within 5% of each other. When results are published, the details of the method used and the type of apparatus should be given, as well as the results obtained in normal controls.

Basal Metabolism

"Basal metabolism" is defined as the metabolic activity present in the morning, twelve to fourteen hours after the last meal, with the subject completely quiet and in the supine position. Determination of the basal metabolism is important in clinical practice. Benedict uses the term "fasting metabolism," and others use the term "resting-fasting value." Krogh showed that metabolism is even lower during sleep, so that basal metabolism does not determine the minimum metabolism, and he therefore suggested the word "standard metabolism." However, the terms "basal metabolism" and "basal metabolic rate" have become deeply rooted in clinical practice. There is a certain justification for this, for the conditions under which the basal metabolism is determined are a convenient point of departure for metabolic experiments, so that the basal metabolism is a base-line for the results of such experiments.

In the study of pathological changes of energy metabolism or of the action of drugs on patients, it is necessary to distinguish in each instance whether the basal metabolism itself is changed or whether events within the metabolic processes are affected. The basal metabolism can be considered "elevated" only when the basal conditions are satisfied during the test - e. g., in thyrotoxicosis or during thyroxin administration. On the other hand, the term "increased basal metabolism" should not be employed for the specific dynamic action of foods or for the increase of metabolism caused by epinephrine. These comments are not merely academic, for by definition basal metabolism reflects the total metabolism, and the few conditions and materials which can alter the basal metabolism also affect the total metabolism. On the other hand, the specific dynamic action of foods and the increased metabolism caused by various drugs are increases which occur in addition to the total metabolism. If there is a change in basal metabolism, one may conclude that there is also a change in the total metabolism. The assumption that a normal basal metabolism indicates a normal total metabolism is also generally made, but rarely with justification.

Normal Basal Metabolism

Harris and Benedict [58] give normal values for basal metabolism, based on the following equations:

$$h = 664.730 + 13.7516 w + 5.0033 s - 6.7550 a \text{ (males)}$$

$$h = 665.0955 + 9.5634 w + 1.8496 s - 4.6756 a \text{ (females),}$$

where h is the heat formed in Calories in 24 hours, w is the weight in kilograms, s is the height in centimeters, and a is the age in years. These values are given in Harris-Benedict tables and are completely valid in the normal individual. They are inaccurate, however, in persons of unusual body build and in the elderly [59, 60].

Rubner [8] demonstrated a close relationship between the metabolic rate and the surface area of the body in warm-blooded animals. Under basal conditions, animals in good nutritional state showed heat production in 24 hours of 11.3 Calories per kilogram (for a horse weighing 441 kg) to 212 Calories per kilogram (for a mouse weighing 18 grams) (see Table 7). When the calories produced were calculated per unit surface area, the values lay between 917 Calories per square meter (rabbits) and 1188 Calories per square meter (mouse). The other warm blooded animals studied showed values between these two extremes (horse, pig, man, dog, goose, chicken). The agreement is very good, since the variations within a single species may be of the order of 10 to 20 percent. The reason for this constancy of basal metabolism is an interesting problem in physiology which is as yet unsolved.

The simplest explanation for the proportional relationship between basal metabolism and surface area would be given by the assumption that the level of basal metabolism is determined by the amount of cooling, which in turn is proportional to the surface area. Regnault and Reiset had already suggested that heat loss is the parameter which determines the total metabolism. Actually, however, the problem is much more complicated. Adaptation to a change of environmental temperature, for example, takes place, not through a change in the basal metabolism, but through changes in body insulation and in regulation of heat loss. Rubner showed that the metabolic rates of two guinea pigs remained proportional to their surface area and remained normal even when they were placed into a temperature of 30° C, a temperature at which difficulties in elimination of heat already appear. Benedict and Benedict [62] found the same results in experimental subjects who were placed into baths at warm temperatures (35 to 38° C). An even more striking example of the lack of relationship between basal metabolism and the external temperature is shown by the experiment of Hösslin [63], who reared two dogs from the same litter at two different environmental temperatures, 32° C and 5° C. The temperature difference between the body of the dog reared in the cold and that of his environment was six times as great as that of the brother dog. The basal metabolism of the cold dog was only 12% higher than that of its brother, but its fur weighed three times as much as that of the warm brother. These studies show that, under normal conditions, the heat output alone is not responsible for the basal metabolic rate. O. Frank and F. Voit [64] found that the basal metabolism of a curarized dog fell when care was not taken, by adjustment of the environmental temperature, to maintain its body temperature at a normal level. These results were at first interpreted as indicating that the basal metabolism is a function of the body mass, especially the musculature. The studies of Hösslin, Benedict and Frank, however, do not contradict the surface area law, for even when one considers differences in insulation, the body mass does not by itself account for the metabolic rate of various large animals. Thus, for a given moderate amount of

heat production per unit body weight, the mouse would require a fat layer of 10 to 20 centimeters to prevent freezing, while a horse would become more and more overheated. Rubner's experiments in animals of various sizes have been repeated by many investigators and, as a result, the "surface area law" is now unequivocally accepted in studies of basal metabolism.

The surface area of a body is proportional to the volume raised to the $\frac{2}{3}$ power:

$$S = k V^{2/3}$$

In this equation, k is a constant which depends on the shape of the body. DuBois used this equation to calculate the surface area of the human body by substituting the weight of the patient for the volume and using a factor for the patient's height (page 29). For comparison of the metabolic rates of different species, the value of the exponent is somewhat greater. Brody [65] gives the value as 0.73, and Kleiber [66] as 0.75:

$$\text{Resting metabolism} = k G^{0.73}$$

It is not clear whether the exponent contains a correction for the generally more elongated stature of large animals or for the metabolism of some tissues which is proportional to the body weight. Newer mathematical studies have given further applications of the surface area law and interesting proofs of the correctness of the exponent 0.73 [66 a].

Since the relationship between surface area and basal metabolism is certain, and the environmental temperature is ruled out as a regulating factor, it must be assumed that the heat production of the tissues differs according to the surface area of an animal. Heat production, which varies with the size of the animal, could be a fundamental property of the tissues, or could be influenced by neurogenic or humoral factors. The relationship between metabolic rate and surface area cannot, however, be explained genetically because of the validity of the surface area law within a given species.

It has long been known that the metabolism of excised tissues in vitro is smaller, the larger the animal from which the tissue is taken, although it has not been possible

Table 7. Average uptake of oxygen by brain and liver slices of 9 animal species compared with the average basal metabolic rate. Partly from data of Krebs [61]

Species	Average body weight (kg)	Oxygen utilization (Q_{O_2})		BMR (Cal)*** in 24 hr per kg body weight (b)	$\frac{a^{**}}{b}$
		Cerebral cortex	Liver (a)		
Mouse	0.038	— 22.9	—	145	—
	0.026		— 19.3	152	0.13
Rat	0.26	— 19.2	— 14.6	92	0.16
Guinea pig	0.44	— 17.4	— 9.5	85	0.11
Rabbit	1.21	— 15.1	— 7.6	57	0.13
Cat	2.75	— 15.5	— 10.2	50	0.20
Dog	18.3	— 14.8	— 10.8	31	0.35
Sheep*	35	— 11.3	— 6.2	27*	0.23
Ox	320	— 12.1	— 3.6	21	0.17
Horse	760	— 10.5	— 2.6	17	0.15

* Of the 5 sheep studied, 3 were young animals. The figure for BMR may therefore be too low (this author's note).

** Author's calculation.

*** From Benedict's Tables.

Table 8. The Relationship of Cytochrome Oxidase Activity to Body Weight [67].
(See remarks under Fig. 4)

Tissue	Species	Units used for enzyme activity	Proportional to
Liver	Mouse, rat, dog	per mg liver-N	weight ^{-0,24}
Skeletal muscle	Rat (65-450 grams)	per mg fresh weight	weight ^{-0,24}
Cardiac muscle	Rat, dog	per mg fresh weight	weight ^{-0,31}
Kidneys	Rat, mouse	per total tissue	weight ^{0,70}

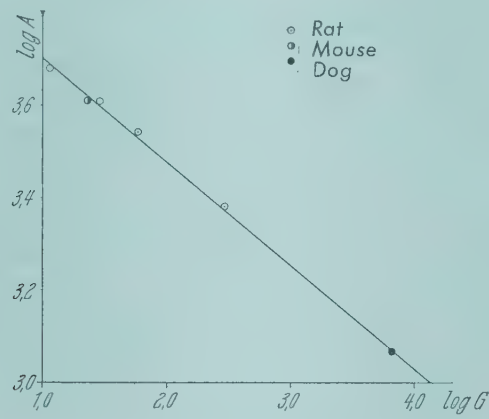


Fig. 4. Relationship between liver cytochrome oxidase and body weight. From Kunkel and Campbell [67].

Abscissa: Logarithm of body weight (G).

Ordinate: Logarithm of cytochrome oxidase activity (A), calculated as the oxygen utilization per mg of liver nitrogen. The equation of the straight line is

$$\log A = 3.934 - 0.237 \log G.$$

Using data the of Kleiber [66], the basal metabolism (BMR) is proportionate to $G^{3/4}$; i. e.,

$$BMR = kG^{3/4}$$

The oxygen utilized per unit weight of tissue (S) is then given by

$$\frac{BMR}{G} = S = \frac{kG^{3/4}}{G} = kG^{-1/4}$$

Taking logarithms,

$$\log S = \log k - 0.25 \log G.$$

This expression corresponds to that obtained experimentally. Assuming that the metabolism of a certain organ represents in all species the same fraction of the basal metabolic rate:

$$\text{organ metabolism} = (BMR)k' = k'k G^{3/4}.$$

to set up experimental conditions sufficiently exact to evaluate this fact quantitatively. Kleiber [66] was the first to show that the oxygen utilization of liver slices of the same weight from rats, rabbits, cattle, sheep, and horses fell to the same degree as the basal metabolism when related to the weight of the animal. Krebs [61] confirmed and extended these observations (Table 7). At the same time, however, he also showed that there is no exact proportionality between the oxygen utilization of slices of other tissues (cerebral cortex, renal cortex, spleen, lungs) and the basal metabolism per unit weight, although here too there seems to be a general tendency in the same direction. As related to the metabolism of the animal as a whole, the oxygen utilization of the liver, of all these organs, plays the greatest role, since the liver is the largest of the organs studied. Of even greater importance for total balance would be

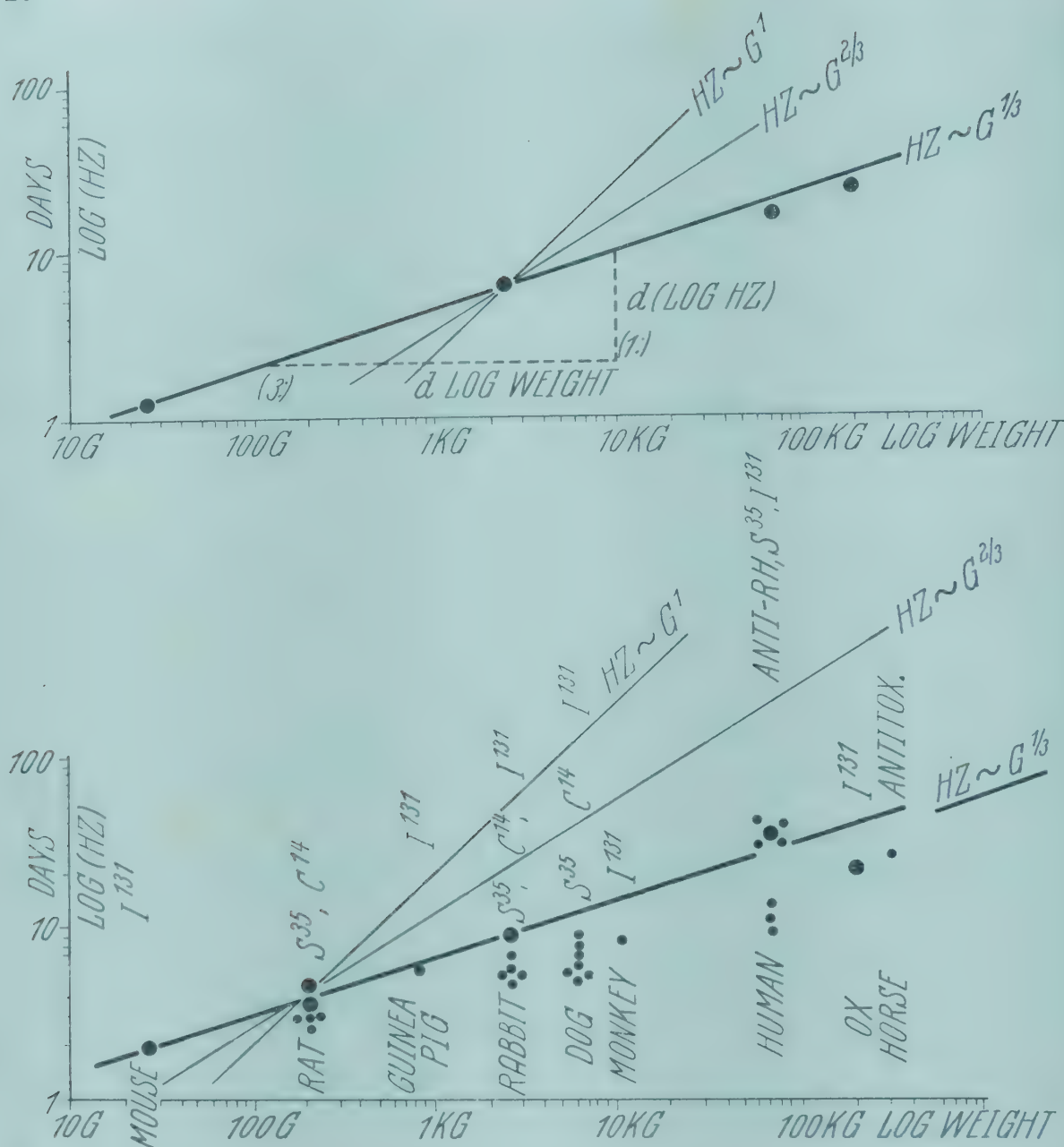


Fig. 5. Relationship between the half-life of the serum proteins and the body weight. From Maurer[69]

(a) Experimental results using I^{131} -albumin.

(b) Data from the literature for variously labeled proteins.

The data can be described as follows: $d(\log HZ) = \frac{1}{3} d(\log G) = d(\log G^{1/3})$
 $\log HZ = \log C + \log G^{1/3} = \log CG^{1/3}$
 $HZ = CG^{1/3}$
 (HZ = Half-life, G = body weight)

Translating the speed of synthesis of serum proteins to the total organism gives

$$HZ = CG^{1/3} = \frac{(\text{amount of total serum protein}) \ln 2}{(\text{rate of synthesis of serum protein})}$$

Assuming that the amount of total serum protein $\approx kG$, the rate of synthesis of serum protein for the total organism becomes $\frac{kG \ln 2}{CG^{1/3}} = KG^{2/3}$. Thus, the rate is proportional to the surface area and the BMR. The equation for the renewal of serum protein per unit weight is

$$\frac{\text{renewal rate}}{G} = \frac{KG^{2/3}}{G} = KG^{-1/3}.$$

the resting metabolism of the muscles, but satisfactory measurements of these values have not yet been made. Kunkel and Campbell [67] showed in mice, rats, and dogs that the activity of cytochrome oxidase is proportional to the basal metabolism even if the measurements are carried out in tissue homogenates instead of in tissue slices. (Cytochrome oxidase is the enzyme responsible for the oxidation of hydrogen liberated during dehydrogenation and is thus responsible for most of oxygen metabolism.) In addition, the tissue content of cytochrome C, which is closely related functionally to cytochrome oxidase, falls in proportion to the basal metabolism calculated on the basis of weight in the following animals: mouse, rabbit, dog, and horse [68]. These findings do not determine whether the basal metabolism is determined by the tissue content of cytochrome oxidase or whether the content of this material becomes adapted to the metabolic requirements of the animal. They at least explain, however, the constancy of basal metabolism in short-term experiments. The values of cytochrome oxidase found in muscle homogenates suggest that the musculature behaves like the liver (Figure 4, Table 8). Besides the in vitro measurements of oxygen utilization in tissue slices and homogenates, the metabolism of certain proteins has been shown to be dependent on the basal metabolism. Maurer [69] has shown that the rate of synthesis and degradation of certain serum proteins is proportional to the basal metabolism in a number of animals. The half-life is noticeably shorter in the mouse than in the rat (Figure 5). It is probable that still other reactions will be found whose magnitude is proportional to the level of basal metabolism.

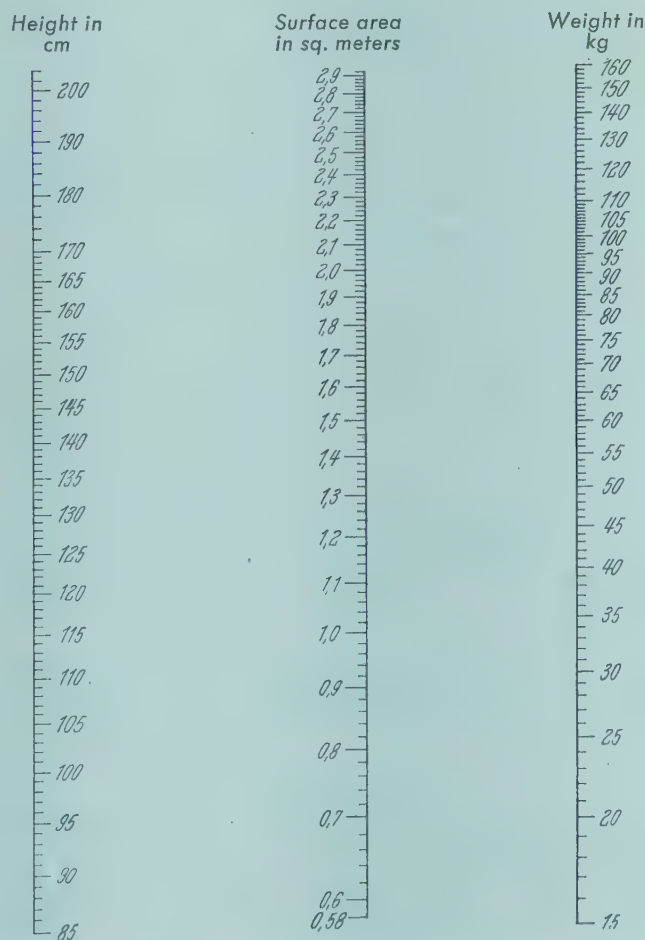


Fig. 6. Nomogram for determining the surface area from the height and weight. Formula of DuBois and DuBois, from the Geigy Scientific Tables, Basel, 1953.

Table 9. Calculation of the Surface Area from the Linear Formula [73, 12]

<i>Head</i>	$0.308 A B$ A = circumference from the tip of the chin to the top of the crown B = circumference from the occiput to the forehead directly over the eyebrows
<i>Arms</i>	$0.611 (F) (G + H + I)$ F = from the tip of the acromion to the lower end of the radius of the outstretched arm G = circumference directly below the axilla H = largest circumference of the forearm I = smallest circumference of the forearm (above the head of the ulna)
<i>Hands</i>	$2.22 J K$ J = volar surface of the distal end of the radius to the tip of the second finger K = circumference of the open hand exclusive of the thumbs at the level of the metacarpophalangeal joints
<i>Trunk</i>	(inclusive of neck, breasts, and external genitalia) $0.703 (L) (M + N)$ L = upper end of the manubrium to the upper end of the symphysis M = circumference of abdomen at the level of the umbilicus N = circumference of the chest at the level of the nipples in the male or immediately above the breasts in the female
<i>Thigh</i>	$0.508 (O) (P + Q)$ O = tip of the greater trochanter to the tip of the patella (legs in adduction) P = circumference directly below the perineum Q = circumference of the hips and the buttocks at the level of the trochanter
<i>Leg</i>	$1.40 R S$ R = sole of the foot to the tip of the patella S = circumference at the tip of the patella
<i>Foot</i>	$1.04 (T) (U + V)$ T = length of foot (great toes) U = circumference of the foot at the basal joint of the small toe V = smallest circumference about the ankle (immediately above the malleoli)

When the body is symmetrical, the constants give the surface area for both sides. Adding the seven values given above gives the total surface area.

These results have not yet been entirely explained. Above all, there is no satisfactory explanation for the proportional relationship between basal metabolism and surface area. The heat output of the animal at the time of study is not a satisfactory explanation for the basal metabolism is independent of the external temperature and of the different types of insulation of various animals (hide, feathers, etc.). However, one can certainly reject hypotheses which relate the basal metabolism to the so-called "active protoplasmic mass" or which explain the high basal metabolic rates of small animals on the basis of a relatively higher proportion of "active tissues."

At first, the concept of using the surface area for the determination of the basal metabolism met with considerable criticism on a number of theoretical grounds. The heat-liberating surface of the body is not identical with the total body surface area (page 33), but this objection was overcome by the use of a proportionality factor. A correction also was made following Pfaundler's objection [70] that there is no such thing as an absolute surface area because of variations in the elasticity of the skin, and for the different temperatures of the surfaces of the trunk and the extremities. A factor can also be found to allow the substitution of some other value, such as the silhouette, for the surface area, provided the substitute is proportional to the surface area [63, 71]. Physiologically, however, the logical value to use is the surface area itself.

The oldest attempt to determine the surface area of a human being was made by John Abernathy in 1793, but the first successful studies were those of Meeh [72]. Meeh's formula for surface area was as follows:

$$12.312 \times (\text{weight in kg})^{2/3} = \text{surface area in dm}^2.$$

The results found by this formula were soon found to be too high and various improved formulas were soon devised. That of DuBois and DuBois [73] is the most accurate:

$$(\text{surface area in cm}^2) = (\text{weight in kg})^{0.425} \times (\text{height in cm})^{0.725} \times 71.84$$

This formula is usually expressed as a nomogram (Figure 6). In addition, DuBois and DuBois have given another, "linear" formula (Table 9). For adults of normal body build, the error of both formulas is $\pm 1.7\%$. The linear formula, however, also permits the determination of the surface area of individuals with unusual body builds, of amputees, of dwarfs, and of cachectic and very obese individuals - all with the same degree of accuracy.

The methods of determining the surface area are extremely accurate, but those factors which relate the basal metabolism to surface area have had to be improved with time. Improvement was accomplished primarily by improved standardization of the methods used and by comparison of large numbers of published series. Bene-

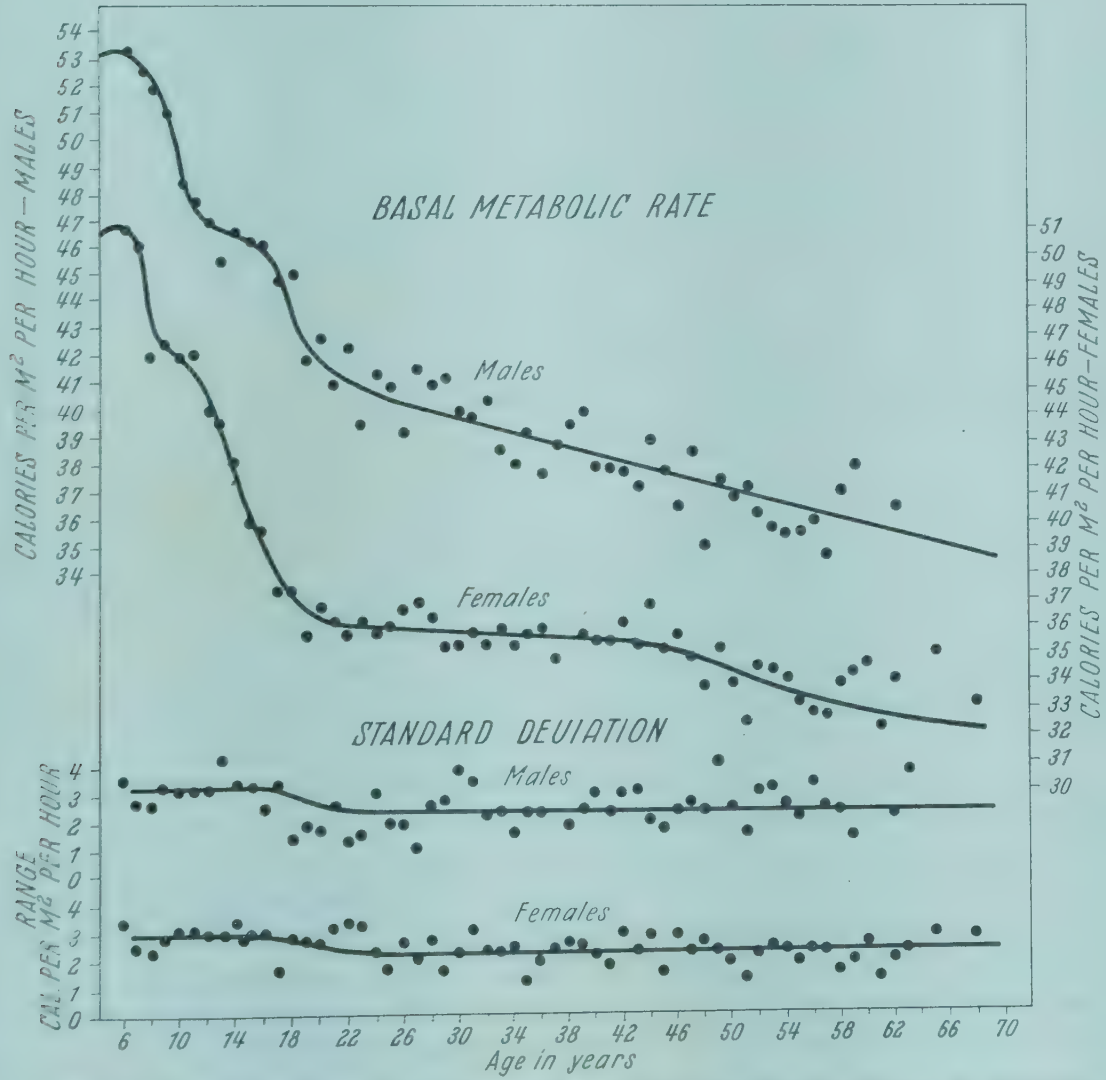


Fig. 7. Average values for basal metabolic rate with the standard deviation at various ages. (From Boothby, Berkson, and Dunn [75].)

Table 10. Basal metabolic rate in calories per square meter of body surface per hour [75, 76, 77]

Age	Males			Females		
	Boothby et al*	Lewis et al**	Fleisch***	Boothby et al*	Lewis et al**	Fleisch***
1	—	—	53.0	—	—	53.0
2	—	56.9	52.4	—	52.9	52.4
3	—	54.5	51.3	—	51.3	51.2
4	—	52.6	50.3	—	49.9	49.8
5	—	51.0	49.3	—	48.4	48.4
6	53.0	49.6	48.3	50.5	46.9	47.0
8	51.5	46.6	46.3	46.7	44.0	43.8
10	48.0	43.6	44.0	45.7	41.4	42.5
12	46.8	41.5	42.5	43.9	39.7	41.3
14	46.4	41.1	42.1	41.1	36.8	39.2
16	45.5	—	41.4	38.6	—	36.9
18	42.9	—	40.6	37.0	—	35.9
20	41.6	—	38.0	36.3	—	35.3
25	40.3		37.5	36.0		35.2
30	39.6		36.8	35.8		35.1
35	38.9		36.5	35.7		35.0
40	38.3		36.3	35.5		34.9
45	37.6		36.2	35.3		34.5
50	37.6		35.8	34.4		33.9
55	36.3		35.4	33.4		33.3
60	35.7		34.9	32.8		32.7
65	35.1		34.4	32.4		32.2
70	34.5		33.8	32.2		31.7
75	33.4		33.2	32.0		31.3
80	—		33.0	—		30.9

* [15]; ** [16]; *** [77].

diet's tables have already been discussed. The standard values of Aub and DuBois [74] were also used and underwent various modifications. Their most modern formulation was made by Boothby, Berkson and Dunn [75] (Figure 7, Table 10). The prediction of the basal metabolism by means of these values together with the surface area formula of DuBois is the most exact of all available methods. Krogh's normal values were obtained under non-clinical conditions (page 22) – preceding diet was free of protein [60] – and are therefore not suitable for the clinical determination of basal metabolism. Lewis, Duval and Iliff [76] gave values for children at various stages of growth. (The values given by the older authors such as Magnus-Levy are generally too high because their “basal” conditions differed from those which we use today.)

The “normal values” of Boothby, Berkson and Dunn were usually obtained as the result of a single test. For patients who have had some practice, these values are about 10% too high; patients who have repeated determinations of “basal” metabolism show a fall, as they become accustomed to the procedure, of about 10% in their “basal” metabolic rate. These results must be remembered in clinical practice. For physiological studies, the normal values given by Fleisch [77] are the most accurate (Table 10).

Factors which Influence the Basal Metabolism

The level of basal metabolism is influenced by a number of different factors. Each individual, however, seems to have a relatively constant basal metabolic rate, as shown by Zuntz et al [78] and DuBois [12] as a result of numerous repeated studies on themselves over long periods of time.

The basal metabolic rate is dependent on the *sex* and the *age*. Basal metabolism is lower in women than in men and falls in both sexes as age increases. In addition to this reduction with age, a further fall occurs at the menopause. The relatively high basal metabolism found below the age of 20 is probably related to growth. In addition to age and sex, other factors have a lesser influence on basal metabolism. Thus, Gessler [79] showed a seasonal variation in the same individual, with reduction in summer and increase in winter. Climatic conditions also influence the basal metabolism: In tropical climates the basal metabolism tends to be lower than in temperate climates [18]. The basal metabolism is lower among Australians, Chinese, Indians, and Syrians, and higher among American Indians, than among white people living in the same environment [12]. People used to manual work generally have a higher basal metabolic rate than sedentary people [80, 81]. The type of diet has no influence on the basal metabolism, if the determination is made at least 14 hours after the last meal. However, the specific dynamic action of a protein-rich meal may sometimes persist after 14 hours, and this fact explains the high basal metabolism of Eskimos. Krogh [60] has shown that a diet particularly poor in proteins can lower the basal metabolism. The basal metabolic rate is often elevated in the last trimester of pregnancy and returns rapidly to normal after delivery. The basal metabolism is somewhat elevated immediately before a menstrual period and is somewhat less than normal during the period. The reasons for all these physiologic changes of the basal metabolism are not known.

With respect to the effect of drugs on the basal metabolism, it must be emphasized that, after a patient has received a drug, his metabolic rate is no longer basal. In general, therapeutic agents do not elevate the basal metabolism itself but merely influence the total metabolism. This distinction is important, for in various cases it can be shown that qualitative alterations are also present such as, for example, increased catabolism of carbohydrates.

Elevated metabolism occurs after the administration of epinephrine [82], caffeine [83], camphor and atropine [84], and often after smoking [85]. There is no increase after alcohol [87]. A number of sedatives, notably morphine and also barbiturates and chloral hydrate, reduce the total metabolism. Morphine and alcohol change the R.Q., alcohol because of its low R. Q. of oxidation (0.67) [87], and morphine by inhibiting the respiratory center with resulting retention of carbon dioxide.

In contrast to these substances, drugs which act on the thyroid glands or which can replace the secretion of these glands influence the basal metabolism proper (page 175). Iodine has no effect on the normal metabolism but can reduce the basal metabolism of hyperthyroid patients [88, 89, 90], and the effect of thiourea, thiouracil, and related antithyroid drugs is even more marked [91]. Iodine affects only metabolism which is increased for thyrogenic reasons, but the antithyroid drugs also reduce normal basal metabolism. Thyroxin and active preparations of the thyroid glands cause an increased metabolism in all individuals [92, 93]. The effect of dinitrophenol is similar [94], but this drug is clinically interdicted because of its severe toxic side effects (hepatic damage, cataracts, agranulocytosis).

Characteristic and often diagnostic changes of basal metabolism occur in a number of disorders which do not affect the thyroid glands (Table 11). These changes are due in part to the influence of the diet and of physical activity, but in part other factors such as hypothermia, hyperthermia, increased synthesis, and pathological growth are also involved. The determination of the basal metabolic rate in heart disease requires especially careful interpretation. If the technic is too simple, the values obtained for oxygen utilization give results which are falsely high. With the exception of febrile endocarditis, cardiac disorders in general show a normal metabolism.

Pituitary extracts act on the thyroid glands by way of thyrotropic hormone to elevate the basal metabolism. Thus, 40% of Cushing's acromegalic patients showed an elevated basal metabolism, but only 25% of his patients showed enlargement of the thyroid. The reduction of basal metabolism following hypophysectomy is the result of a diminution in the amount of thyrotropic hormone produced (pituitary myxedema).

Table 11. Basal metabolic rate in disease states
(Based on at least 20 cases in the literature)

Diagnosis	Basal metabolic rate		
	Below — 15%	— 15% to + 15%	over + 15%
Normal	—	99*	1*
Neurasthenia	1	97	2
Mental disorders	5	92	3
Epilepsy	13	85	2
Encephalitis (16 cases)	—	87	13
Obesity	4	92	4
Diabetes	17	75	8
Acromegaly	3	57	40
Hypopituitarism	85	15	—
Addison's disease	8	84	8
Heart disease	2	94	4
Hypertension	1	86	13
Endocarditis	1	88	11
Cardiac neurosis	—	96	4
Renal disorders	5	88	7
Intestinal disorders	3	94	3
Gynecological disorders	4	92	4
Dermatological disorders	1	97	2
Arthritides	3	92	5
Malignant tumors	11	67	22
Leukemias (all types)	—	23	77
Polycythemia (2 cases)	—	1 case	1 case
Anemias (various forms)	2	83	15
Hyperthyroidism	—	7	93
Hyperthyroidism, relapse after operation	—	11	89
Toxic adenoma of thyroid	—	32	68
Nontoxic adenoma of thyroid	—	100	—
Colloid goiter	3	94	3
Myxedema	83	17	—
Postoperative myxedema	80	20	—
Cretinism	63	37	—
Thyroiditis	9	50	41
Thyroid carcinoma	5	71	23

* percentage of cases.

Regulation of Body Temperature

The maintenance of the normal body temperature demands that the production of heat equal the loss of heat. If heat production increases, the body temperature is elevated; if heat loss increases, the body temperature falls. The temperature balance is part of the homeothermia which is characteristic of the body.

Teleologically, one can distinguish between "useful" and "useless" production of heat. The same amount of heat, however, may be either "useful" or "useless" depending on the environmental surroundings. Thus, the production of heat in strenuous work is beneficial in cold weather but can be useless in hot weather. Often, heat production belongs to neither category, and sometimes it is not certain to which category heat production belongs (for example in fever).

The amounts of heat formed and heat lost are determined by the activity and surroundings of an organism. If there is a discrepancy between these two figures, regulatory processes must come into play to reestablish equilibrium. In basal metabolism, as well as during work, the determining factor is heat production, for neither a reduction nor an increase of the environmental temperature alters the metabolic rate if these changes are in the range of so-called "indifferent temperature." It is only the temperature of the skin which is affected by the environmental temperature and which maintains the temperature difference between body surface and environment at a constant level, thus controlling the elimination of heat.

The chief regulator of heat balance is the elimination of heat from the body. The heat formed is the heat loss of the events of life, so that the production of heat can be influenced only by reduction or increase of bodily activity. The mechanisms for heat elimination, on the other hand, adapt to the environment and are thus primarily responsible for the maintenance of the body temperature.

Heat Elimination

The body has three mechanisms for the elimination of heat: convection, radiation, and evaporation. One or another of these mechanisms plays the most important role according to the status of the body and its environment.

Convection is the transfer of heat by conduction. Physically, the molecules of the skin convey to the molecules of the environment impulses which correspond to its warmth. The reverse occurs if the environment is warmer than the skin. The amount of heat given off by convection depends on the surface area, the surface temperature, and the physical properties of the environment (temperature, heat-conductance, heat capacity, and motion). Thus, one feels warmer in calm than in wind, in the air than in water; and, of two floors of equal warmth, the one with the poorer heat-conductance feels warmer. Depending on the temperature of the skin, the various parts of the body have different roles in heat loss by convection, but this fact cannot be readily quantitated.

The most important of the mechanisms for the elimination of heat is *radiation*, which accounts for over 50% of the total heat loss of the body. The radiant surfaces comprise only 80 to 85% of the surface area of the body, for surfaces which lie opposite each other, such as the upper arms and the axillae, do not radiate heat. The radiant surface in the sitting position is less (71–75%) than in the standing position (91–95%). Physically speaking, the human skin, independent of its actual color, is a black radiating body; that is, it absorbs (or radiates) all infra-red rays equally well. The exchange of heat by radiation therefore depends only on the temperature difference and the area of the radiant surface. We constantly give off heat rays to the four

cooler walls of our rooms while we take up heat rays from the heating system. The air between the parts of the body involved in heat radiation does not become warm in the process, for only air of high humidity absorbs heat to some degree and thus interferes with the elimination of heat.

The mechanisms of radiation and convection taken together are often considered the "sensible heat loss." The heat loss by radiation in a man with surface area of 1.8 meters at room temperature approximates 7 Calories per hour per degree of temperature difference between the skin and the radiating surfaces of the surroundings. When the environment is very hot, such a man can take up up to 10 Calories per degree [95]. Radiation and convection have in common the fact that, according to the environmental temperature, they can lead either to heat loss or to heat uptake.

The amount of heat lost is adapted to the requirements of the body by changes in the temperature of the skin. The skin temperature depends on two factors, the flow of blood through the skin and the thickness of the subcutaneous layer of fat. The blood

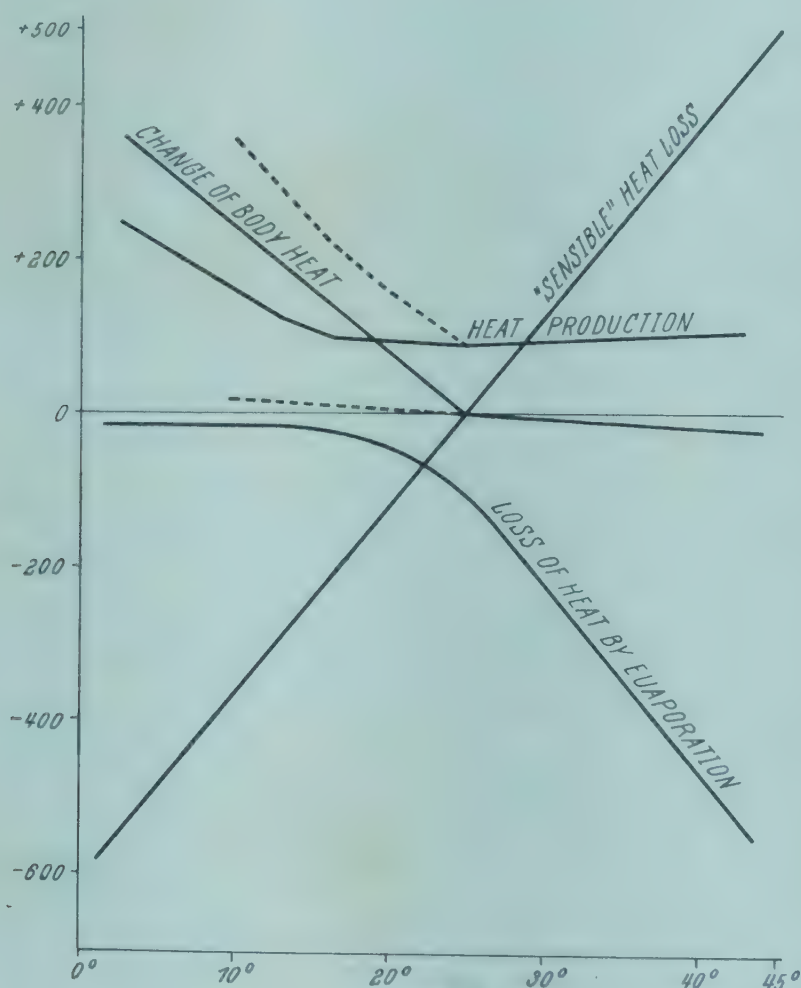


Fig. 8. Diagram to show regulation of internal body temperature of a man resting in the sun.

Prepared from the data of Adolph and Molnar [98].

Abscissa: environmental temperature.

Ordinate: heat exchanged (calories per hour).

The solid lines represent the first hour; the dotted lines represent the situation after attainment of the final temperatures. At still lower temperatures, heat production by shivering is not sufficient to maintain the internal temperature of the body. Clothing, muscular work, shade, and wind may shift the individual curves to the right or the left, but without changing their shape. See also [25] and [26].

flow is dependent on the temperature of the blood flowing through the brain, on reflex changes of skin temperature due to changes in brain and spinal cord centers, and on direct reactions of the blood vessels to changes in the external temperature [96]. In the hand, for example, the blood flow can fall from 30 cc/100 cc tissue per minute at 37° C to 0.15 cc at 0° C. The adjustment of blood flow to environmental conditions can take 30 to 45 minutes or, in exceptional cases, hours. The blood vessels of internal organs – for example, the coronary arteries – often take part in the reflex changes of blood flow. A high external temperature increases the cardiac output. The blood volume also changes with the environmental temperature in accordance with the changes in the skin blood flow. When the temperature is hot, the capillaries become wide and the blood thin; when the temperature is cold, hemoconcentration occurs. These changes are under neurogenic control. Deep transection of the spinal cord destroys this control mechanism and leads to impaired temperature control. Little need be said concerning the insulating action of fat tissue. Obese individuals can stand the cold better than people of normal weight and, by the same token, obese people show more rapid increase in temperature during increased heat production (e. g., during work).

When heat production is large or when the external temperature is high, *evaporation* is the predominant mechanism of heat loss. Evaporation is also known as “insensible perspiration” or “insensible heat loss.” The effect of evaporation on heat balance is always negative or, when the humidity of the air is 100%, zero. When the external temperature increases, so that the sensible heat loss becomes smaller, the loss of heat by evaporation increases. While the sensible loss of heat remains bound to the height of the body temperature, the heat loss by evaporation can reach a level several times normal by insensible perspiration and later by actual sweating. Evaporation depends, of course, on the humidity of the air, and at a given temperature the insensible decrease of weight and accompanying loss of heat by evaporation are smaller the higher the humidity of the surrounding air [97]. This physical fact is especially marked in so-called comfortable surroundings (light clothing at rest at 30° C), when the temperature and moisture of the skin show little change. When the environmental temperature becomes lower or higher, other factors enter into the picture, vasoconstriction on the one hand and sweating on the other.

The production of sweat is controlled by a special center in the brain which is stimulated by the temperature of the blood. The center sends out impulses which reach the sweat glands by way of the sympathetic nervous system; the sweating of a portion of the body can be eliminated by denervation. The sweating center also reacts to muscular work, before the blood is heated. In addition to the center in the brain, there seem to be other centers in the spinal cord, for after transection of the cord the part of the body below the level of transection may still show sweating. On the other hand, local heat does not produce sweating. Sweating is independent of the blood flow. “Cold sweat” has less to do, as a rule, with heat regulation than with psychic factors. When the intake of food is plentiful and good, facial sweating may occur, as in the shining fat faces of gourmands in books and paintings. Vagus stimulants, especially pilocarpine, cause increased sweating, and atropine inhibits sweating. Sympathicomimetic drugs such as epinephrine and ergotoxin have no influence on sweating.

The role of clothing in heat exchange depends on the type of heat exchange present. The air trapped beneath the clothes is a good insulator and reduces the loss of heat by convection, but this is normally small in any case. The loss of heat by radiation is not influenced by ordinary light clothing, for heat flow can take place through the

thickness of the clothing and is proportional to the heat production [95]. The situation is different when very heavy clothing or insulating materials are worn. Here the conduction of heat through the material is limited and this limitation can protect from both heat loss and overheating. In hot weather, clothing can increase the uptake of heat by enlarging the surface area. The influence of clothing on evaporation depends on the type of clothing. The insensible perspiration is certainly reduced by clothing when the humidity is high. On the other hand, if sweating occurs, the clothing increases the surfaces of evaporation. This influence is so important that the level of heat tolerance in tropical climates is higher for people wearing light, absorptive clothing than for those who go about naked.

Heat Production

All heat formed in the body is ultimately derived from chemical reactions. At first, therefore, the regulation of the body temperature was considered chemical in nature, while regulation of body temperature by adjustment of heat output was called physical. However, only a portion of the heat formed in the body is derived directly from chemical reactions, while another portion is the end result of other forms of energy such as, for example, the mechanical energy of the work of the cardiovascular system. Instead of using the terms "chemical" and "physical" regulation of temperature, therefore, it is better to speak of heat production and heat elimination.

Food intake is followed by an increased production of heat. This increase depends on the type and amount of food ingested as well as on the nutritional state of the individual. The increased heat production during digestion was named by Rubner the "specific dynamic action" of the food ingested [99] and was explained "as a loss of energy, a simple direct production of heat in the process of conversion of the foodstuff to a form suitable for cellular nutrition" [100]. The specific dynamic action is expressed as a percentage of the caloric value of the ingested food. Expressed in terms of the preexisting metabolic rate, usually the basal metabolic rate, the specific dynamic action is not physiologically significant. The greatest specific dynamic action is that of the proteins, which is always greater than 10% and sometimes as high as 40%. Amino acids have the same effect as proteins, so that it is believed that the increased heat production is due to the processes of deamination. The specific dynamic action of the carbohydrates is smaller (about 10% of the caloric value), and that of the fats is the least (2 to 2.5%). The mean specific dynamic action of an average meal in a person of normal nutrition is 5 to 6 percent. In malnourished individuals, the value may be lower, probably because of a tendency to replenish the depleted nitrogen depots of the body. When the ingested meal is large, however, high values are found for the specific dynamic action both in the normal and in the overnourished individual. Prolonged feeding of protein in experimental animals leads to prolonged elevation of the metabolic rate. Rubner called this finding the "secondary specific dynamic action," and Grafe and Graham coined the term "Luxuskonsumption" (literally, luxury consumption of food) on the basis of these findings. Actually, both concepts do not differ from the action of the primary specific dynamic effect and need not be discussed separately. Grafe's hypothesis [101] that, in too rich dietary intake, the body protects itself against the deposition of fat by the phenomenon of "Luxuskonsumption," could not be confirmed by Benedict [102]. DuBois has also shown that the opposite hypothesis, that there is a reduction of the specific dynamic action in obesity, is untrue. The endocrine organs have no influence on the specific dynamic action.

The intestinal epithelium is not the site of the specific dynamic action, for parenteral as well as oral feeding result in the same caloric response. Against heat formation due to absorption there is also the finding that, in a phlorhizinized animal, oral feeding of carbohydrate is not followed by an increase in metabolism. For proteins, it has been shown that the site of the specific dynamic action is probably the liver. The hepatectomized animal thus fails to show any increase in metabolism following the administration of glycine or alanine [103], and feeding casein to rats increases the oxygen utilization of liver slices in vitro. There is probably no special site for the specific dynamic action of fats and carbohydrates, and the effect should be looked upon as an expression of the total body metabolism.

Among the causes of physiological increase of the rate of metabolism, work – and, more exactly, muscular work – is quantitatively the most important. Each muscular movement increases the processes of oxidation. For example, even when just the arm is raised, the calorimeter shows increased heat production (DuBois). The mechanical efficiency of muscle is 20 to 30%. When a muscle contracts, 20 to 25% of the total expenditure of energy can be converted into mechanical energy, and under favorable conditions as much as 30%. In disorders of the thyroid gland, the efficiency is less and, as a result, thyroid patients produce more heat under the same conditions of work than normal subjects. Patients with an increased basal metabolic rate for non-thyroid reasons, however, have normal muscular efficiency. In patients with cardio-pulmonary disorders, the efficiency is poor because of the increased respiration and circulation necessary for the transport of oxygen (Table 12).

Table 12. The energy required to walk in certain diseases.
From Boothby and Sandiford [104]
$$\text{Calories required} = \frac{(\text{Total calories/hours}) - (\text{Calories/hours at bedrest})}{(\text{Kilometers/hours}) \times (\text{Body weight})}$$

Diagnosis	Variation of mean BMR from ideal %	Calories per km per kg body weight	Variation from normal %
Normal	— 10	1.20	—
Diabetes mellitus	— 13	1.09	— 10
Chronic myelocytic leukemia	+ 20	1.10	— 8
Chronic lymphocytic leukemia	+ 65	1.19	— 1
Hypertension	+ 4	1.43	+ 19
Myxedema	— 28	1.36	+ 13*
Thyroid carcinoma	+ 43	2.31	+ 93
Hyperthyroidism	+ 52	2.24	+ 87

* In contrast to this result, see page 159.

When a muscle contracts isometrically, all the energy is given off in the form of heat. On the other hand, if we assume that the efficiency of work, for example using a bicycle ergometer, is 25% *, and gas analyses show that this work requires 300 Calories more than the basal metabolism, then $\frac{25}{100} \times 300$ Calories are transformed into other forms of energy in the ergometer. At the same time, $\frac{75}{100} \times 300$ Calories are

* Efficiency = $\frac{\text{mechanical work accomplished}}{\text{energy liberated above resting requirements}} \times 100$.

converted by the body into heat and the body must give off this heat by means of its heat-regulating mechanisms. If the regulation of heat loss is impaired, the body would become $75 \times \frac{300}{100} \times \frac{1}{75} = 3^{\circ}$ Centigrade warmer (calculated for a 75 kg man with a specific heat of 1).

Centers of Heat Regulation

The regulation of body temperature by *heat loss* (physical regulation of heat) may take place, depending on the circumstances, either by "heat-saving" or by "increased loss of heat." The ability to save heat by reduction of the blood flow through the skin is less, however, than the ability to give off an increased amount of heat under thermal loads. The regulation of body temperature by the adjustment of the amount of heat given off is therefore especially important when the environment is hot and when there is increased production of heat in the body. The regulation of body temperature by *varying the production of heat* (chemical regulation of heat) is unable to save heat and can only effect an increased production of heat. Muscular effort seems to be the most important source of heat in times of need and it is self-evident that this heat production occurs only insofar as necessary. However, when the surroundings become cold, the necessary additional heat can also be produced by other means such as the specific dynamic action or an increase in basal oxygen consumption of the tissues. Under such conditions, little or no muscular activity occurs. Hart, Heroux and Depocas showed that rats acclimated to the cold do not shiver at 6°C , while warmth-acclimated animals do shiver, indicating that heat formation may be due almost exclusively to factors other than muscular activity [167].

The temperature-regulating centers of the body are located in the hypothalamus (Figure 9) and respond to afferent stimuli from the skin as well as to the temperature of the blood which flows through them. Excision of the cerebral hemispheres, the thalamus, and the corpus striatum therefore does not cause any disturbance of the body temperature (Figure 9, line 1). If, however, the thalamus is excised or if, in otherwise intact animals, a section is made between the lowermost cervical segments and the corpora quadrigemini (Figure 9, line 2), temperature control is lost at least temporarily and the animal becomes poikilothermic. Section of the upper thoracic segments above the exit of the sympathetic nerves affects heat-regulation only by affecting the heat output; there is no effect on the production of heat. Animals whose cervical cord is sectioned lose the ability to have fever; animals with section of the cord at the thoracic level retain this ability.

In recent years, the temperature-regulating centers have been localized more exactly [105], chiefly as the result of Ranson's work in cats and monkeys [106]. Ranson showed that there is a center in the supraoptic and preoptic region of the hypothalamus, warming of which leads to increased heat output of the animal. If this region is damaged, temperature regulation is lost even when the increase in environmental temperature is so small that it would normally be insignificant, so that hyperthermia results. Since the control of body temperature during heat chiefly involves the regulation of heat loss, it must be assumed that the supraoptic and preoptic centers are responsible for the regulation of heat output (physical regulation of heat). Further support for this assumption is given by the fact that animals with lesions in these areas do not lose their ability to withstand cold. This ability to maintain the normal body temperature in the cold is lost in animals with lesions in the caudal portion of the lateral hypothalamus, and it is here that the centers for the

regulation of heat production must be sought (chemical regulation of heat). Several workers, notably Thauer [168], maintain that, in addition to the control of temperature by the hypothalamic centers, there must also be some sort of peripheral regulation, since animals with transection of the upper cervical segments or with excision of the hypothalamus regain the ability to control temperature a few days after the operation, although to a limited extent only, because of the paralysis of most muscles.

The results of clinical physiological studies of intracranial disorders show [107] that disturbances in the region of the supraoptic nucleus may cause hyperthermia, while those in the posterior hypothalamus cause a tendency to hypothermia. The temperature-regulating centers are therefore probably located in the same place in both man and monkeys. However, as is well known in experimental neurophysiology, about any experimental lesion there are other areas of damage which appear initially but are later resolved. Acute functional deficits can therefore be correlated with the anatomic picture only to a limited degree. This fact explains to some extent various contradictory statements in the literature concerning neurogenic control of temperature. Hypothermic states of central origin, and hibernation, are associated with an elevation of the serum level of magnesium.

When there is prolonged reduction of ambient temperature, the secretion of thyroid hormone is increased and, at the same time, an animal with an intact pituitary gland develops hyperplasia of the thyroid glands. These reactions, however, may only be an expression of the increased requirement for hormones in general due to the increased total metabolism of the organism. The calorogenic effect of epinephrine is probably too transient to contribute any sizeable amount of heat when the requirement for heat is significantly increased.

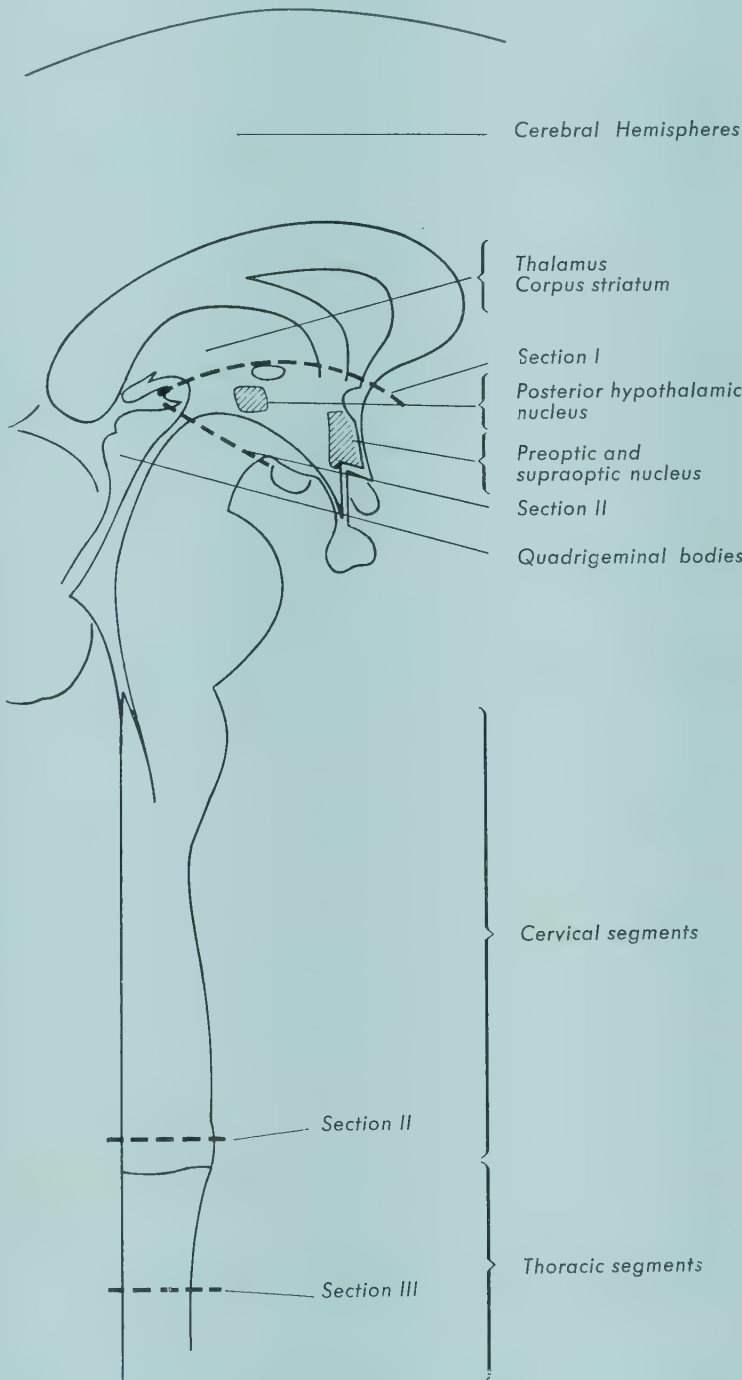


Fig. 9. Effect of certain transections of the central nervous system on the temperature regulation mechanism. See text.

Fever

During the nineteenth century there was constant dispute as to whether fever is only a disturbance of the output of heat with constant formation of heat, or whether, in addition to a disturbance of heat loss, there is also an increase in the amount of heat produced. At the turn of the century, the presence of an increased metabolism during fever in man and in animals was clearly demonstrated by Krehl and Matthes, but it was incorrectly concluded that the elevated metabolism was the cause of the fever. The problem was solved by DuBois by simultaneous determination of the heat output and the oxygen consumption. Barr and DuBois [108] showed that, at the beginning of an attack of malaria, the oxidative processes are increased as measured by gas analysis, but despite this fact, the output of heat, measured calorimetrically, is normal. The result of this discrepancy is an elevation of temperature. DuBois and his co-workers later found that this mechanism of fever is not specific for malaria but occurs generally. When fever subsides, the reverse phenomena occur; i. e., there is increased output of heat (calorimetrically) with normal heat production (gas analysis). The metabolic rate is increased in fever, as can be anticipated from van't Hoff's law that the higher the temperature, the greater the speed of chemical reactions. On the basis of personal experiments and data from the literature, DuBois calculated the temperature coefficient of the metabolic rate in fever as 2.3 [109] — i. e., a theoretical temperature rise of 10°C increases the metabolic rate 2.3 times.

Fever, an increase in temperature, results from a discrepancy in the regulation of heat production and heat loss. However, the maintenance of the elevated body temperature is largely due to adaptation of heat loss to the new temperature level. The amount of heat produced in fever is not so large as to exceed the body's ability to eliminate it; the mechanisms of heat loss are capable of eliminating much larger amounts of heat produced by muscular effort or by the specific dynamic action of a protein-rich meal. The increased metabolism in fever is not the cause but rather the result or expression of the increased reaction temperature in the tissues. Fever is thus less a disturbance of metabolism than a disturbance of heat regulation. The stimulus which produces a rise of temperature is quite unknown. In infections, protein catabolic processes, and similar disturbances, many authors consider the stimulus to be a central toxic action. Beeson thus extracted a pyrogenic factor from leukocytes [109a, 109 b]. Certain other types of fever, such as those due to salt or to thirst, cannot be explained in this way. Barbour [110] therefore postulated that the action of pyrogenic substances occurs peripherally, producing hemoconcentration, which in turn has a further pyrogenic effect. Increased muscular activity can be the cause of a rapid rise in temperature. Chills, which are muscular contractions occurring during rapid temperature rise, are evidence of this fact. The increased production of heat during a maintained fever ("febris continua"), however, must be looked upon as an elevation of the basal metabolic rate.

Fever causes changes in the body metabolism, especially changes in the body water, salts, and proteins, but without producing harmful after effects. In a few cases, such as the artificial production of malaria, fever may be of therapeutic value, but usually it plays no role in the treatment or cure of disease.

The metabolic changes which occur when body metabolism is lowered are the opposite of those of fever. Under certain conditions, it is possible, by means of ice-packs, to reduce the temperature of human beings and other warm-blooded animals to 25°C or 20°C without causing death. Breathing, pulse, blood pressure, and basal metabo-

lism are markedly reduced. Artificial hypothermia, because of its effect on tissue metabolism, is used in the surgical treatment of certain disorders because it permits the temporary interruption of blood supply even to susceptible tissues [110 a]. The state induced may be likened in many respects to hibernation, but it is not yet clear to what extent the comparison is valid. Under certain conditions, hibernating animals (hamsters) can be restored to life (by warming) after cooling to a temperature below 0° C and partial freezing [110 b]. Detailed discussion of the problem of hibernation does not belong in a book on human metabolism [110 c]. The designation “hibernetic” which is sometimes applied to chlorpromazine and certain similar medications is erroneous, for they cause neither a significant fall of oxygen utilization nor a fall of body temperature.

Disorders Due to Cold and Heat

The last war, with battles in the Arctic as well as in tropical climates, gave impetus to extensive research into the reactions of the body to cold and heat. Figure 10 shows the reaction to *cold*. Exposure to a cold environment results in prompt fall of the skin temperature, with the temperature of the extremities very close to those of the environment. In this “last retreat” of the regulation of heat output, the skin temperature is determined almost exclusively by diffusion of heat from the interior of the body and no longer by blood flow [98]. When the cooling process reaches the deep tissues, so that the rectal temperature falls below 36° C, the cold becomes unbearable but neither deadly nor dangerous. Under these extreme conditions, the insulating value of the subcutaneous fatty layer is especially important. (Bordier [111] had already shown in 1898 that fatty tissue, because of its low conduction of heat, can maintain twice as great gradients of heat as the musculature.) Re-warming of cold skin occurs with the speed of cooling only on the trunk: at the extremities, re-warming goes on very slowly, and the skin temperature of the extremities may remain below that of the environment for hours.

When maximal constriction of blood vessels has taken place and the skin temperature has reached its lowest point (in Figure 10, after about 10 minutes), the final

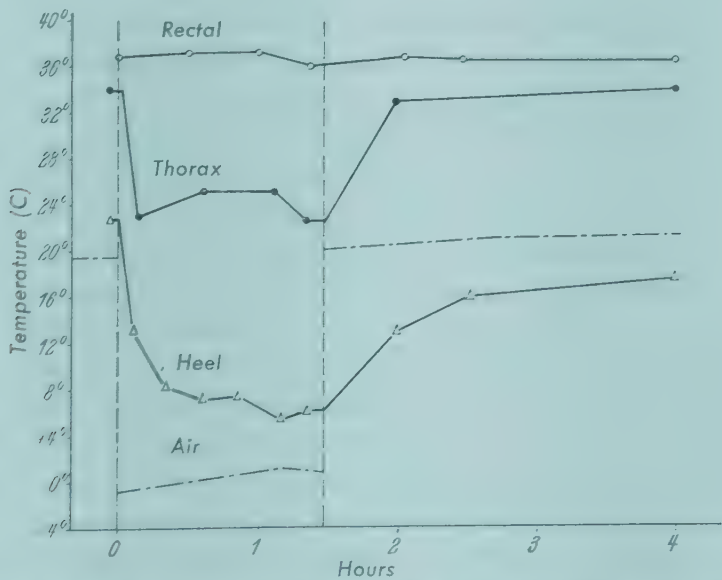


Fig. 10. Maintenance of rectal temperature and skin temperature in an experimental subject who was first fully clothed in a room, then naked in the open air, and finally lightly covered in a room. Experiment of Adolph and Molnar [98].

gradient between internal and external temperatures is established and there can be no further saving of heat by regulation of heat output. The heat which is still given off under these conditions must be constantly replaced by production of new heat, so that temperature by heat-production takes over the maintenance of body temperature. In severe cold and in non-acclimated individuals, the increased heat production occurs predominantly as the result of rapidly repeated muscular contractions; i. e., chills. The resulting amount of energy is very large: cases have been described in which more than 200 Calories per square meter of surface area per hour were produced. This amount of heat corresponds to that produced by effort maintained for 3 to 4 hours, up to the point of exhaustion. As a rule, exhaustion also limits the ability of the conscious individual to withstand cold. When the musculature can no longer produce heat, cooling begins, with its usual clinical sequels [98].

The pulse of the chilled person is elevated, more so in the sitting position than when he is lying down. The blood pressure is normal or slightly elevated. The blood is somewhat thickened (the hemoglobin goes up, the hematocrit is elevated, and the proteins are increased, all from 10 to 15 percent of the original values), probably as a result of narrowing of the blood vessels. The thickening of the blood overburdens the extravascular extracellular space and leads to a cold diuresis with a relatively high specific gravity of urine (over 1.009), a result of the excretion of electrolytes dissolved in the plasma. Cold diuresis is more marked in patients who are lying down than in those who sit, but in the latter edema of the ankles is frequent. If the skin temperature falls below 16° C, pain occurs. This does not last long, however, for analgesia soon follows, although hyperesthesia may still persist.

The *impairment* of temperature regulation in the cold is manifested in a number of ways. Sweating of hands and axillae is an uncertain sign. The fatigue which follows the severe effort of chills is often no longer noticed. The most reliable objective sign is the change in the psychic state of the subject. Severely chilled people become dull and unable to concentrate, are confused and uncertain and, in extreme chilling, are disoriented as to time and place. After re-warming, they have amnesia for the period of disorientation. The psychic changes cause a vicious cycle brought on by chilling: somnolence, which is part of the chilling syndrome, leads to reduced muscular production of heat, thus to further chilling, and ultimately to death. In contrast to heat stroke, there is no sudden destruction of the defense mechanisms of the body but rather a gradual reduction to lower and lower levels of metabolism and life. The body finally stops functioning with a fall of blood pressure and cardiac arrhythmia [98 a].

Treatment is based on the cause of the chilling. Acute cases, as seen when a person is forced to remain in cold water (in accidents and during war), must be managed by hot baths. Warming of the patient by means of hot air or arc lights takes too long, and hot or cold massage is without rationale or effect. The prognosis becomes apparent rapidly. During recovery, high body temperatures and confusion may occur. In contrast to acute cases, therapy of prolonged chilling is poorly understood. It is probable that the surviving individual under the circumstances has become accustomed to the new level of metabolism, and re-warming should therefore be slow. Experiences with re-warming after artificially induced hypothermia would appear to support this concept.

The physiology of acclimatization to cold is as yet poorly understood. Scholander and his co-workers showed that white Norwegian men living for weeks in a cold climate and sleeping with little protection at 3° to 5° C acquired the ability to keep their heat production 50% above basal metabolic levels while asleep [169], while Australian aborigines in similar surroundings, lying naked between small fires, keep

warm by reducing the body surface temperature [170]. The latter reaction to a cold environment implies an increased tolerance to surface cooling and suggests that the compensatory heat formation in acclimated white people is at least partly induced by surface cooling. Increased physical fitness is also capable of modifying the responses to cold [171], and it appears possible that some of the effects of cold acclimatization are the result of physical training which usually accompanies acclimatization.

The local clinical sequelae of exposure to cold are frostbite and immersion foot. In frostbite, there is damage to the tissues with prolonged analgesia of the involved portions of the skin, thromboses, and sequelae of thrombosis (ischemic necrosis, gangrene, abscesses). Marked chilling of an extremity, even when it does not cause local frostbite, results in a well-defined physiopathological syndrome ("immersion foot"). The sequelae of immersion foot include severe edema with anesthesia of the skin, and neuromuscular disturbances, especially disturbances in gait. As the disease progresses, degeneration of nerves and muscles occurs and interstitial fibrosis ultimately takes place. Montgomery [112] considers immersion foot as a diffuse inflammation without ischemic thrombosis, while he considers frostbite as a combination of the effect of freezing with the effects of thrombosis which occurs shortly after thawing. In most conditions due to chilling, there is a combination of immersion foot and local trauma, infection of the skin, and frostbite. The word "trench foot" is used for this combination. The clinical picture is varied. Re-warming may lead to hyperemia which may last for weeks, to purpuric lesions of the skin, and to gangrene. Shock, severe pain, abscesses, cellulitis, thrombosis, and ankylosis may occur as complications. Hematuria and albuminuria, with tubular damage by hemoglobin casts, are frequent.

Therapy should be conservative: rest, elevation of the affected extremity, and careful asepsis. Except in cases of pure immersion foot, anticoagulation with heparin is indicated. Antibiotics should be used as necessary. Rubbing of the skin, massage, and the use of arc lights are contraindicated because of possible damage to the involved tissues. The prognosis is generally good, although sensitivity to heat and cold may remain for years. Persistent pain is usually due to osteoporosis, which can be seen on x-ray.

When the environmental temperature rises above the comfort level, heat output alone is responsible for regulating the body temperature. There is no adaptive fall of heat production. Indeed, at high temperatures the basal metabolism rises and the output of heat is still more taxed. As long as the surrounding temperature is less than the skin temperature, both sensible and insensible heat loss occur. When the surrounding temperature becomes higher than that of the skin, evaporation must take care of the entire output of heat. The pathological changes due to elevated temperatures must therefore be due either to sweating or to cessation of sweating. As a rule, under such conditions, the increased production of heat by physical activity and the high surrounding temperature and humidity work together. Three clinical pictures can be differentiated: heat cramps, heat prostration, and heat stroke [113].

Heat cramps, which occur as an occupational disorder in stokers and miners, affect the muscles of the extremities and sometimes those of the abdominal wall. The disorder is caused by disturbances in the electrolyte content of the body as a result of the loss of sodium chloride in the sweat. In severe physical activity, the secretion of sweat can amount to 850 grams per hour, even in prolonged studies. If the amount of salt lost in this process is not replaced by the ingestion of fluids containing sodium chloride, the amount of sodium chloride lost can be considerable even though the chloride content of sweat is low (between 5 and 60 meq/liter) (113 a). The plasma then shows reduced levels of sodium and chloride. Therapy consists of absolute bedrest and the administration of

sodium chloride, parenterally if necessary, until the painful cramps stop [114]. In many cases, 1,500 cc. of physiological saline may be enough. Sedatives and morphine are usually without effect. Prophylactically, the administration of salt prevents heat cramps.

Heat prostration is usually produced by local overheating, especially by exposing the head to strong sunlight. The prodromal symptoms are weakness, nausea, vertigo, and headache. The disorder begins suddenly. The blood pressure falls, the pulse is rapid, and the skin is cold and moist. There is no impairment of temperature regulation, for the internal body temperature and the skin temperature are normal and the secretion of sweat is not disturbed. Therapy consists of rest and the oral administration of fluids. Infusions are seldom necessary.

Heat stroke (pyrexia) is diagnosed by the rectal temperature, which may be elevated to 43°C (109.4°F). The skin is hot and dry. The cause of the disorder is probably cessation of sweating [115]. Heat stroke occurs chiefly in elderly persons. In addition to the fever and the dry skin, the first symptoms are headache, vertigo, and abdominal colic. If coma, cramps, petechiae, shock, or a temperature over 41°C (106°F) supervene, the prognosis is poor. Shock is an especially poor prognostic sign. Later changes include icterus, nephrosis, bronchopneumonia, electroencephalographic changes, and mental disturbances. Anatomically, there may be hemorrhages in the brain, lungs, endocardium, and intestinal tract. Therapy is urgent. Its first aim is to return the body temperature to normal. This is most easily accomplished by cold baths until the body temperature has fallen to 38°C (100°F). Placing the patient in the shade is ineffective and reduces his chances for survival. For a few hours, the body temperature must be controlled and regulated by the use of appropriate baths, until the body itself can maintain its temperature at normal levels. Drugs must be used with care, for the high body temperature may have caused cellular damage. The use of sedatives is contraindicated except for cramps. Infusions are permissible only if there is constant control of the venous pressure and of the concentrations of sodium, potassium, and chloride in the serum [116]. Oxygen is beneficial. Careful observation is necessary to uncover latent damage to the body tissues. The ability to sweat does not return for several days, so that heat must be carefully avoided.

Heat damage can be prevented by preventing exposure to sources of heat. Even short daily periods of training (30 minutes) in elevated temperatures produce acclimatization to heat within a week. Such training results in an altered blood volume, in increased ability to sweat, and in improved reactions of the circulation. In addition to training in hot temperatures, all types of physical training and good nutrition improve the ability to withstand heat. Light clothing makes high temperatures more bearable. Insufficient sleep, poor health, and the use of alcohol [115] predispose to the heat disorders. Patients who are unable to sweat are especially liable. The sweat glands are lacking in congenital ectodermal dysplasia. In addition, there is an acquired form of anhidrosis which occurs after prolonged fevers such as typhoid. There also seems to be a psychogenic inability to sweat. The "exhaustion" of the sweat glands, especially of the trunk, has already been discussed.

Comments on Nutrition

The dietetics of the various disturbances of metabolism are discussed in the appropriate chapters of this book. At this point, we shall merely discuss dietetics in general [115 a]. The ability of the healthy individual to adapt to all possible diets is considerable, and the search for an "ideal" diet is therefore close to impossible [115 b].

For this reason, recommendations of special diets cannot really be contradicted as long as the observe they basic rules of caloric intake and of absorption. People have a tendency, when given free choice, to choose a high-protein diet as far as possible economically. When 13% to 15% of the total calories are given in the form of protein, especially animal protein, the protein minimum of the diet is easily covered. However, many peoples eat diets with consiiderably more protein without apparent damage. In addition, the diet contains fat which supplies 15% to 20% of the calories, and the rest of the calories come from carbohydrates. A high-fat diet reduces the life expectancy of the experimental animal [115 c] and may also be damaging in human beings, especially in the presence of certain disorders such as diabetes (page 288). Damage to blood vessels may also occur (chapter 11). In addition to the requirements for calories and proteins, there is a daily minimum requirement of other substances,

Table 13. The daily requirement of essential dietary elements.*

Mean values during normal life. The requirement of children depends on the age and must be estimated.

	Adults	Children Aged 12 years	Infants Aged 12 months	Remarks
<i>Vitamins</i>				
Vitamin A	5,000 units	4,500 units	1,500 units	Requirement of pantothenic acid and vit. B ₆ is not known.
Vitamin B				
Thiamin	1.8 mg	1.2 mg	0.4 mg	
Riboflavin	2.7 mg	1.8 mg	0.6 mg	
Nicotinic acid	18.0 mg	12.0 mg	4.0 mg	
Pantothenic acid	5-10 mg	5-10 mg		
Pyridoxin	2.0 mg			
B ₁₂	0.5-1 μg			
Folic acid	0.1-0.5 mg			
Vitamin C	75 mg	75 mg	30 mg	
Vitamin D	400-800 units	400-800 units	400-800 units	
Vitamin E	10 mg			
Biotin	0.01-0.05 mg			
Vitamin K	2 mg			
<i>Amino acids</i>				
L-isoleucine	0.7 grams		126	mg per kg body weight
L-leucine	1.1 grams		150	
L-lysine	0.8 grams		103	
L-methionine	1.1 grams		45	
L-phenylalanine	1.1 grams		90	
L-threonine	0.5 grams		87	
L-tryptophane	0.35 grams		22	
L-valine	0.8 grams		106	
<i>Minerals</i>				
Iodine	10-100 μg			Since iron is absorbed in limited amounts, 10 times the listed amounts must be ingested.
Iron	1.2-2.2 mg	2.2 mg	1.2 mg	
Calcium	0.8 grams	1.0 grams	1.0 grams	

* The values given differ only slightly from those given in the "Recommended Dietary Allowances" of the National Academy of Sciences, 1958.

Table 14. Approximate caloric requirements in various body states*
(Collected from the literature)

State	Energy turn- over during the state	Total energy requirement for an 8-hour working day, 70 kg man. BMR calculated for 24 hours = total BMR
	As factor of BMR	
Sleep	$0.9 \times \text{BMR}$	
Sitting, Reading	$1.3 \times \text{BMR}$	
Mending	$1.4 \times \text{BMR}$	
Knitting	$1.5 \times \text{BMR}$	
Dressmaking	$1.8 \times \text{BMR}$	
Typing, Ironing, Washing	$1.9 \times \text{BMR}$	$1.7 \times \text{Tot. BMR}$
Housewife, Office worker		$1.4 \times \text{Tot. BMR}$
Outpatient		$1.2 \times \text{Tot. BMR}$
Bed patient		
	Cal. per kg body weight per hour	
"Light" manual labor (tailor, bookbinder, shoemaker)	2.5	$1.4 \times \frac{16}{24} \times \text{Tot. BMR} + 8 \times 70 \times 2.5$ $\approx 0.9 \text{ Tot. BMR} + 1400 \text{ cal}$
"Moderate" manual labor (lock- smith, cabinet maker, brick- layer, painter)	3.5	$0.9 \times \text{Tot. BMR} + 2000 \text{ cal}$
"Heavy" work (farmer, wood- cutter, stone-mason, miner)	4.5-7	$0.9 \times \text{Tot. BMR} + 2500-4000 \text{ cal}$
Standing	1.5	
Walking (2.5 mph)	3.0	
Walking (3.8 mph)	4.3	
Walking (down steps)	5.2	
Walking (up steps)	16.0	
Running (5.3 mph)	8.0	

* Individual variations are, of course, tremendous.

especially vitamins and minerals. Table 13 gives the daily requirements of such substances. The requirements in special physiological states, such as pregnancy, and in various metabolic disturbances, are discussed in the appropriate chapters.

The calculation of the caloric requirements of an individual can be made from various data which give the number of calories required for a given amount of work. This procedure ignores the size and weight of the body. The type of data given in Table 14 are more accurate; these give the energy turnover of individual events in relation to the basal metabolism. If the nourishment required is to be calculated from the energy turnover, a factor of 10% must be added to the average specific dynamic action. For a printer, for example, the daily caloric needs would be calculated as follows:

$$(\text{basal metabolism per day}) \times 1.8 \times 1.1.$$

The Food and Agriculture Organization of the United Nations has published the basic principles for the determination of the caloric requirements of a given population [117]. According to these, a 25-year old man weighing 75 kg and living in

temperate zones (e. g., Munich or London), and who performs work of average intensity, requires 3,550 Calories per day:

average daily requirement (males) at age 25 = $152 \times (\text{weight in kg})^{0.73}$
average daily requirement (females) at age 25 = $123.4 \times (\text{weight in kg})^{0.73}$

Correction factors for these formulas include a 7.5% reduction for each increase of ten years in age and a 5% increase for each 10° C fall of the average yearly temperature. During the last trimester of pregnancy an additional 450 Calories must be added per day, and during the postpartum period (lactation) an additional 1,000 Calories. The daily requirement for children is especially difficult to determine. Table 15 gives the values as calculated by the United Nations Commission.

Table 15. Average Daily Caloric Requirement during the Growth Years [117]

Under 12 months	110 calories/kg
1- 3 years	1200 calories
4- 6 years	1600 calories
7- 9 years	2000 calories
10-12 years	2500 calories
13-15 years females	2600 calories
males	3200 calories
16-19 years females	2400 calories*
males	3800 calories*

* Body weight 65 kg.

Very little is known concerning a general diet during growth. Apparently, provided that the minimum requirements of essential nutrients is met, the growing body does well with any reasonable diet.

In the first year of life, vitamins should be added to the food, preferably as one of the modern tasty preparations which contain all essential vitamins including vitamin D. In mammals, the prevention of infection, even subclinical infection, is important for proper growth. In this connection, in a series of premature twins and triplets, those siblings which weighed less were given 50 mg of Aureomycin per kg body weight and showed more rapid growth than the untreated siblings (29.5 grams/day as opposed to 18 grams/day). At the end of the study, the treated siblings weighed more than the untreated [118]. Similar observations have been made in animal husbandry: when given Aureomycin, the growth of more poorly developed animals surpassed that of their siblings, which had previously grown more rapidly. In the United States, Aureomycin is added to animal feed to stimulate growth and deposition of fat. The small doses used do not affect the normal intestinal flora but eliminate pathological organisms, do not act by increasing the value of the food but rather by increasing the appetite by preventing infection. Chickens raised under sterile conditions show maximal growth without antibiotic additives, and the first generation of chickens in a completely new coop also shows optimal growth which cannot be raised by Aureomycin.

The metabolism during fever always leads to increased breakdown of protein and increased need for protein. As a rule, the degradation of protein is proportional in such instances to the total metabolism. However, when the fever is high, marked catabolism of protein occurs which cannot be compensated for even by a high-caloric diet and a high nitrogen intake. As Shorr has shown [120], strict bed rest results, even in completely healthy individuals, in an increased breakdown of proteins with increased excretion of sulfur, potassium, calcium, and phosphorus, probably as a result of hypo-

Table 16. Components of Various Foods (per 100 grams)

	Protein (grams)	Fat (grams)	Carbohy- drate (grams)	Calories
<i>Meat</i>				
Goose, duck	16.0	28.6-31.5	0	321-349
Chicken	21.6	2.7	0	111
Rabbit, venison	20.0-21.0	5.0- 6.0	0	129-134
Beef, veal, lamb, lean	15.5-32.2	9.0-13.0	0	159-230
moderately fat	11.0-19.5	11.3-31.0	0	230-341
Pork, moderately fat	16.4	25.0	0	291
Ham, moderately fat, raw	15.2	31.0	0	340
Bacon, fat	3.9	85.0	0	781
<i>Sausages</i>				
Salami	23.9	36.8	0	427
Liverwurst	16.7	20.6	0	260
Frankfurters	15.2	14.1	0	201
Beef sausages	16.0	28.0	0	316
Pork sausages	10.8	44.8	0	446
<i>Fish</i>				
Smoked eel	18.6	27.8	0	325
Fresh herring	19.0	6.7	0	136
Fresh codfish	16.5	0.4	0	70
Haddock	16.8	0.3	0	74
Sardines in oil	21.1	27.0	0	331
Oysters	6.0	1.2	0	50
Lobster, crabs, crawfish	16.2	1.9	0	86
<i>Fats</i>				
Butter	0.6	81.0	0	716
Margarine, baking fat, lard	0	100.0	0	884
<i>Eggs</i>				
Raw whole egg	12.8	11.5	0.7	158
Raw yolk	16.3	31.9	0.7	355
Raw white	10.8	0	1.0	47
<i>Milk and Milk Products</i>				
Cow's milk	3.39	3.97	4.94	69
Buttermilk	3.5	0.5	4.8	36
Heavy cream (35%)	2.3	35.0	3.0	336
Light cream (20%)	2.5	20.0	4.0	206
Goat's milk	3.76	4.07	4.64	71
Camembert cheese	19.7	25.2	0	306
Swiss cheese	28.6	31.3	2	404
Limburger cheese	23.5	32.4	1	390
Roquefort cheese	21.7	33.2	1	390
<i>Other foods</i>				
Dark rye bread	6.4	3.4	51.7	263
White bread	8.5	2.0	52.0	260
Biscuits	9.8	9.9	73.5	422
Noodles	13.0	1.4	73.9	360
Polished rice	7.6	0.3	79.4	351
Refined sugar	0	0	100.0	387
Bee's honey	0.3	0	79.5	319
Milk chocolate	6.0	33.5	54	542

	Protein (grams)	Fat (grams)	Carbohy- drate (grams)	Calories
<i>Vegetables</i>				
Potatoes	2.0	0.1	19.1	85
Carrots, turnips, cabbage	1.1-2.1	0.2	6.2-9.1	29-40
Cauliflower	1.6-2.4	0.1-0.2	4.9-5.7	25-31
Spinach	2.2	0.3	3.9	22
Lettuce	1.0	0.3	4.0	23
Dried peas and beans	22.5-25.7	1-1.5	59.2	270-320
<i>Fruits</i>				
Grapes	0.8	0.4	16.7	74
All other fresh fruit	0.5-1.4	0.2-0.6	8.1-16	39-66
Dates, figs	2.2	0.6	75	314
Nuts	3.4-6.7	1.9-4.1	33	213-380
<i>Alcoholic beverages</i>	Alcohol (%)			
Beer	3.4-4.4	0	4.0	50
Rum	44	0	0	312
Wine	7.5	0	0.1	53
Red wine	15.0	0	14.0	163
Brandy and spirits	40.0	0	0	250

plasia of the bones and musculature because of inactivity. The increase in nitrogen excretion averages ten grams of nitrogen per week, corresponding to 65 grams of protein or 300 to 350 grams of meat.

In general, the sick patient should receive an easily digestible diet of low specific dynamic action. The composition of such a diet should correspond to that of the normal diet, and it should be made as appetizing as possible. The administration of a high-caloric diet is often very difficult in patients with fever; ice-cold food is usually readily accepted and should receive especial consideration, especially in the form of large amounts of high-caloric ice cream. Foods with low satisfying value and with high caloric content are especially indicated in patients with poor appetite; foods with low caloric content but which fill one easily, in people who overeat.

The value of the individual foodstuffs with regard to heat production, work, and growth is not the same. For the same amount of muscular activity, for example, 100 calories from carbohydrate equal 112 calories from fat. For the maintenance of body weight, however, fat and carbohydrate have equal value. Protein, on the other hand, has little value in this regard. Alcohol in general can only spare heat. In general, the speed with which foodstuffs enter the metabolic processes is also of importance. Thus, only carbohydrates are able to supply the energy required in certain types of sports.

The healthy body, as a rule, "chooses what it requires." The physician should therefore never disturb the normal diet of the healthy individual except, at best, to supplement it on occasion, and this is also true for the amount of fluid intake. People who lead a hectic, overactive life and who therefore skip meals, however, must be counseled by their physicians. The special danger of quick, skimpy meals often consists of the fact that they merely cover the caloric requirement without including vitamins and minerals. When special diets are necessary, the individual taste of the patient must guide the physician within clinical considerations. Dietary instructions must be simple and clear, and within the means of the patient. They should also be given to the person who actually prepares the food and, of course, they must be followed by the patient.

Obesity
Definition and Measurement

In obesity, the body contains an abnormally large amount of fat, with a resulting increase of the body weight above the ideal weight for the individual.

Since both obesity and extreme leanness reduce life expectancy, the "ideal weight" can be defined as that for which the life expectancy is greatest (page 64). Climate also makes certain demands on the thickness of the fat layer and can therefore influence the ideal weight. Another problem is the determination of the individual ideal weight from simple anthropometric studies. Population statistics give only average values and these are generally too high; in general, too, they ignore the factor of stature. Tables of ideal weight are thus not easy to prepare, and various formulas can be used with equal reliability. Broca's formula is the simplest:

$$\text{ideal weight (kg)} = \text{length (cm)} - 100.$$

Depending on the height, the values obtained by this formula are either good or too high. Bornhardt's formula is better:

$$\text{ideal weight (kg)} = \frac{\text{length (cm)} \times \text{mean chest circumference (cm)}}{240}$$

This formula at least considers the width of the body. After growth has stopped, no correction should be made for the age of the individual until senescence, when the ideal weight decreases to some extent. On the other hand the amount of physical activity is important and must be taken into account (page 52), as illustrated by the greater weight of the large muscle masses of laborers and athletes.

When obesity is present, its cause must be established. The differential diagnosis of obesity from cardiac and renal edema is usually simple. When both water retention and obesity are present in the same person, as in congestive heart failure in fat people and in myxedema, the differentiation may be difficult. Diagnosis in such cases depends on careful measurements and on the results of therapeutic trials.

The thickness of a skin fold depends on the thickness of the subcutaneous fat, the overlying cutis being relatively constant. Normally, the subcutaneous layer of fat comprises half of the total body fat. Measuring the thickness of the skin is therefore a good way to determine the mass of body fat and thus gives a good control for comparisons of the actual and the ideal weights. Such measurements were first made by Oeder [121] and Lauter [122], and more recently by Keys [123]. Keys suggests that

Table 17. Average thickness of the skin folds of 83 subjects.
From Newman and White [124]
The thickness of the skin folds is twice the thickness of the skin.

Location of skin fold	Thickness (mm)	Standard deviation
Arm	12.5	5.0
Chest	14.5	6.9
Abdomen	18.6	7.9

the best measurements can be made at the middle of the upper arm posteriorly, at the trunk in the mid-axillary lines above the iliac crests, and at the back below the scapulae (Table 17). There is good agreement between the values obtained by differ-

11.6 93 34

ent investigators, with a discrepancy of less than 10%. Nylin [125] described an x-ray method for measuring the thickness of the skin. Besides judging whether overweight is due to obesity, skin fold measurements have a special place in the study of disturbances of fat distribution. Edwards has described patients with lipodystrophy and diffuse symmetrical lipomatoses in whom reducing diets resulted in symmetrical

Table 18. Specific gravity of various body and tissue components in man at 36-37° C [123]

Body fat	0.9007
Edema fluid	1.002
Fat-free cadaver	1.09
Normal young subject	1.063
Extra tissue in obesity (62% fat, 14% extra-cellular fluid, 24% cellular material)	0.9478

reduction of body fat from both fat-rich and fat-poor subcutaneous areas, so that there was no improvement in the abnormal distribution of fat [126].

Recent measurements of the density of certain body tissues, the density of the total body (Table 18), and "water spaces" have allowed the development of a formula to calculate the proportion of fat in the body from the weight of the body in air, the weight under water, and the determination of the thiocyanate space:

$$F = (4.149/D) - 0.198 T - 3.717.$$

Here, F is the fat content of the body as a fraction of 1; D is the density of the body, and T is the thiocyanate space. The fat content of a hypothetical person in whom D = 1.063 and T = 0.22 would therefore be

$$F = 3.90 - 0.044 - 3.717 = 0.14 = 14\%.$$

As long as there is no edema, a simplified formula can be used:

$$F = (4.201/D) - 3.813.$$

For the same patient,

$$F = 3.95 - 3.813 = 0.14 \text{ or } 14\%.$$

For a fat person, with D = 1.02 and T = 0.22,

$$F = 4.07 - 3.76 = 0.31 \text{ (31\%)}, \text{ or}$$
$$F = 4.11 - 3.81 = 0.30 \text{ (30\%)}.$$

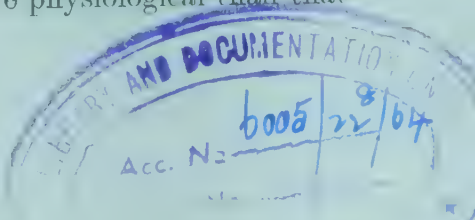
For a person of the same weight, but with edema, in whom D = 1.02 and T = 0.35

$$F = 4.07 - 0.069 - 3.717 = 0.28 \text{ (28\%)}.$$

The calculation of the fat content of the body by determining density depends on the different specific weights of fat and thinner body substances. On the other hand, the measurement of body fat by measuring the body water uses the fact that fat and water are not miscible - i. e., exclude each other. As a result, the relative proportion of body water to total body mass is smaller, the fatter the individual. (The determination of body water can be made with heavy water (DHO), radioactive water (THO), or urea, or, most simply, with antipyrine.) The extracellular fluid space must be taken into account (= 0.7 T), giving the equation

$$F = 1 - 1.563 A + 0.349 T,$$

where A is the antipyrine space [123]. This equation is more physiological than that



used elsewhere, $F = \frac{A}{0.718}$, which does not take into account the water content of fatty tissue. In the normal person, assuming $A = 0.61$, the equation becomes

$$F = 1 - 0.953 + 0.077 = 0.12 \text{ (12\%)}$$

Finally, the fat may be measured by specific fat-soluble substances. Only fat-soluble gases have been of practical value until now, and it has been possible to show that determinations made in experimental animals using nitrogen or cyclopropane show good agreement with chemical methods of measuring fat in the cadaver [127].

The determination of the amount of fat has an extensive application. Chemical analysis of the cadaver has become unnecessary, and large anthropometric series may be obtained in healthy living persons. These measurements have shown that even under widely divergent conditions the body of the female contains more fat than that of the male. Comparison of fat measurements with other anthropological measurements shows, with a high degree of probability, that there is no such thing as a "fat somatotype," e. g., pyknic individuals with tendency to obesity. The body fat can also be studied during changes of body weight, as in re-feeding after malnutrition. It can be shown that an increase of body weight which exceeds a certain weekly amount is due to the formation of fat, which indicates that the ability of the body to form parenchymal cells is limited. Excessively rapid re-feeding following malnutrition is therefore undesirable.

Friedrich von Müller [128] showed many years ago that under certain circumstances the body weight cannot be used as an index of the fat content of the body: reducing diet, starvation, recovery following starvation, and convalescence. In general, however, the body weight is a good measure of the body fat. Keys gives the results of Brozek (Table 19) as evidence that the body weight is a poor index of body fat, but from the standpoint of the clinician it is a fact that the body weight can indeed be used as long as one considers the effect of bodily activity on the ideal weight.

Table 19. Body fat under different conditions of activity [129]

The experimental subjects were all business and professional men. The "active" group spent their free time in recreational activities involving physical exertion; the "inactive group" led a more or less sedentary life.

	"Active"	"Inactive"
Age in years	52.9	52.2
Height in cm	176.1	176.2
Weight in kg	81.5	78.3
Specific gravity	1.0493	1.0435
Amount of body fat	24.3%	27.3%

Pathogenesis of Obesity

A generalized increase of body fat occurs when the intake of food is greater than the output of energy in the form of heat and work. The excess of ingested food is stored as fat, because the body cannot excrete it. This is the only manner in which obesity can develop. As long as a person becomes fat or remains fat on a given diet, he is eating more than he needs. The law of the conservation of energy is the basis of any discussion of obesity and of rational therapy. Energy which is taken into the body is

either used up or stored, and this is true both for patients with hypothalamic disorders as well as for people who habitually overeat. It is thus impractical to distinguish between exogenous and endogenous obesity. The curious fact about obesity is not that it exists, but that it is not more prevalent. The normal person and, even more, the healthy wild animal are astonishingly able to maintain their body weight in the face of marked variations in the dietary intake and in the energy requirements. This ability is lost in obesity.

The general statement of energy balance of the body may be depicted as follows: Food ingested — Fat stored = Basal metabolism + specific-dynamic action + caloric content of the excreta + work + heat production by work + change in heat content. It is immediately evident that when the intake of food is constant, reduction of each value on the right side of the above equation causes increased storage of fat. When the right side is constant, the storage of fat can become zero or even negative when the food intake falls.

The basal metabolic rate, as determined by the surface area, is normal in obesity. Grafe [130] and Boothby and Sandiford [131] showed this in most of their patients. A few patients show reduced basal metabolism (4% in Boothby and Sandiford's series, 3 of 180 patients in Grafe's), and a few show slightly increased basal metabolism. The same is true of any random population of patients.

A diminution of the specific dynamic action of food was at one time thought to be a possible cause of obesity, especially of "pituitary" obesity. Actually, however, the increase of metabolism after the ingestion of protein is the same in normal and in obese persons. However, if one expresses the increase in metabolism after a meal as a percentage of the basal rate, such a meal causes a smaller rise in obese than in normal persons. Such a calculation, however, is inadmissible, for the specific dynamic action does not increase the basal metabolism but is in addition to the basal rate. Furthermore, the magnitude of the specific dynamic action depends, as already discussed, not on the basal metabolism of the patient, but on the amount of protein ingested. Grafe introduced the concept of "Luxuskonsumption," by which excess food is thought to be converted to heat by simple oxidation, thus preventing its deposition as fat. This work has not been confirmed by subsequent studies. The prolonged increase in metabolism which occurs after the continuous administration of a high-protein diet is due entirely to the specific dynamic action. If the prolongation persists beyond fourteen hours, determination of the basal metabolism in the usual way may falsely suggest that the basal metabolism is elevated.

Reduced excretion of oxidizable proteins and fats, or increased absorption of food, would make the energy balance positive. Neuenschwander-Lemmer [132] showed, however, that the oxidative value of the excreta of obese individuals is normal.

There are no abnormalities in the caloric requirements of muscular activity in obese persons, and the efficiency is normal. The total caloric requirement for a given effort, however, is increased, a result of the increase of body weight. This increased energy requirement increases with increasing body weight until the output side of the balance is equal to the intake of calories.

After a high-caloric meal, the skin temperature of the normal individual rises more than that of the obese individual. In the normal, therefore, the mechanism for loss of heat seems to be more efficient than that in the fat person. Actually, in the obese person, the heat formed as a result of specific dynamic action is first retained in the subcutaneous fat, which has a relatively high capacity for heat, before the skin itself becomes warm. For this reason, as compared to the normal, the fat body retains

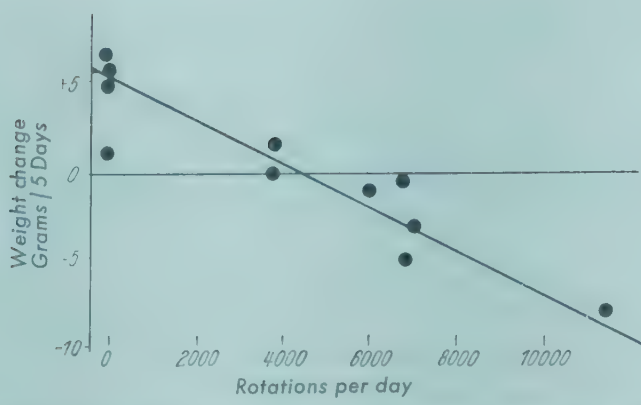


Fig. 11. Negative correlation between activity and change in weight of female rats on a constant diet in a constant temperature room (from Brobeck [133]). Each dot represents one experimental animal.

Abseissa: activity measured as the rotations of a treadmill per day.
Ordinate: weight.

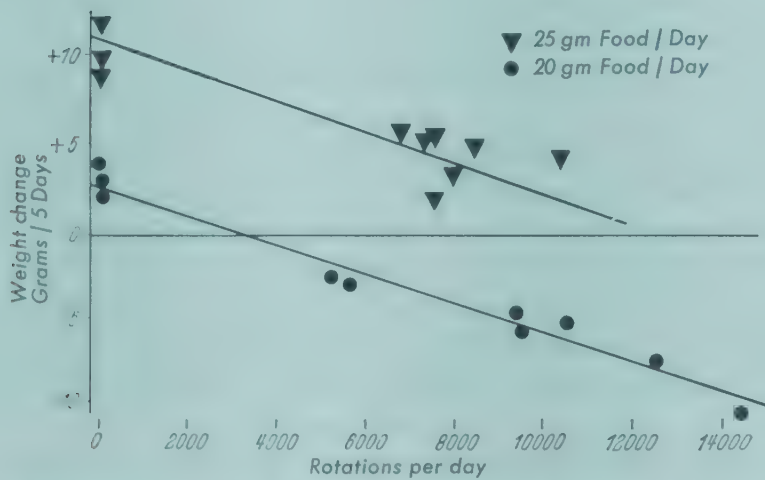


Fig. 12. Negative correlation between activity and change in weight of female rats in a constant temperature room but with varying amounts of food intake. From Brobeck [133].

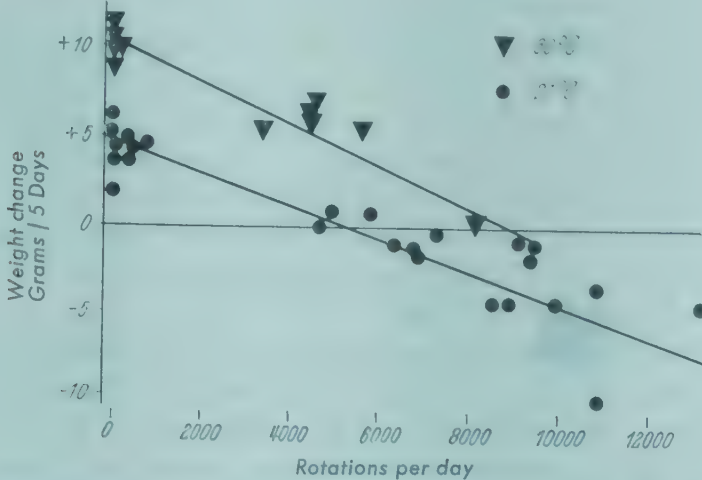


Fig. 13. Negative correlation between activity and change in weight of female rats on a standard diet but under varying environmental temperatures. From Brobeck [133].

excessive heat for a somewhat longer period of time and gives it off only gradually. This is the same mechanism which makes obese individuals more resistant to lower temperatures and more sensitive to elevation of temperature.

The importance of work, environmental temperature, and energy storage within the body in the form of protein, carbohydrate, and especially fat in the regulation of the body weight has been shown in detail by Brobeck [133]. This author placed rats into rooms with constant temperature and air ventilation and compared the intake of food, activity, and body weight. In the presence of constant food intake, there was a negative correlation between activity and increase of weight (Figure 11). When the intake of food was increased, the curve of this relationship shifted to show increase of weight (Figure 12). When activity and environmental temperature were constant, an increased amount of food resulted in increased weight gain. Increasing the surrounding temperature from 21 to 30° C had the same effect (Figure 13). "The described relationships are in full agreement with the principles of thermodynamics and may be predicted from the calorimetric studies and determinations of oxygen utilization by Rubner, Benedict, Lusk, DuBois, and others" (Brobeck). Compared with the usual calorimetric experiment, these studies permit the observation of total energy turnover over a relatively long period time while the animals eat, drink, move, rest, wake, and sleep.

The constancy of body weight implies a sensitive regulating mechanism which controls the normal impulses of hunger and appetite. Clinical observations also seem to imply an urge to physical activity after heavy meals. When obesity is present, there is a disturbance of these regulatory mechanisms.

The size of the appetite can be considered only in relation to the body needs. Obese patients do not always eat to excess: in older people who lead a very quiet and sedentary life, amounts of food which would barely be adequate for younger active adults may be sufficient to cause obesity. Appetite (appetite is synonymous with hunger) is not a simple reflex which follows a certain definite stimulus, but may occur after a number of different stimuli. The size of the appetite depends on several factors, especially the urge to eat and the feeling of satiety. These mechanisms are activated by various stimuli, among which the level of blood glucose is especially important, as shown by the fact that an infusion of glucose can stop hunger contractions of the stomach. J. Mayer showed that glucose is especially important in the regulation of the appetite ("glucostatic theory"). However, it is a fact that the stomach is not necessary for normal caloric balance, for gastrectomized patients maintain their body weight, and satiety follows jejunal tube feeding. Hence, the mechanisms which produce hunger and the instinct to eat must also manifest themselves at organs other than the stomach. Adolph [134] showed that the "dilution" of a diet with substances devoid of caloric value has no influence on the caloric intake, so that mere filling up of the intestine does not control hunger. It is likely that the metabolic state of certain cells in equilibrium with the circulating metabolites has to do with regulation of appetite, but it is not known whether these cells are present within a "hunger center" or are receptors for reflexes.

In civilized people, food is often ingested without the prior occurrence of hunger, and in this case only the feeling of satiety prevents excessive indulgence. Partial satiety with a certain foodstuff must be differentiated from total satiety. A diet of great variety may be both valuable and dangerous, valuable if a person tends to eat too little, dangerous if he tends to eat too much. In addition, there is a specific appetite for almost every foodstuff.

It is customary to consider increased appetite, endocrine factors, and metabolic disturbances as possible causes for the altered caloric balance in obesity. The instinct for food can be measured only by the need for food, so that "increased appetite" and "disturbed caloric balance" cannot be divorced from each other. In obesity, the increase of appetite in excess of the actual need is always of pathogenetic importance, but this is not the fundamental etiology of obesity. Various psychogenic, neurogenic, metabolic, genetic, and endocrine factors may be involved in this "fundamental" etiology. Occasionally, several factors operate at the same time in a given patient, so that the differentiation between primary and secondary effects can be difficult or even impossible.

Experimental studies of obese patients rarely give any clue as to pathogenesis even if the findings in the obese person are different from those in the normal, for it can never be determined if these findings are the cause or the effect of the obesity. Pathogenetically, only those changes are of importance which precede the obesity or persist after weight returns to normal.

The relation between obesity and the central nervous system is seen in hypothalamic obesity. Erdheim was the first to show that tumors of the floor of the third ventricle produce obesity, even when the hypophysis itself is not involved. This finding has been confirmed repeatedly and experimental lesions in the midbrain have been shown to produce obesity.

Bilateral damage to the posterior hypothalamus in the dog interrupts the nerves which proceed caudad. Obesity follows concomitant with the retrograde degeneration of the cells of the paraventricular nuclei. If the nuclei do not degenerate, obesity does not occur. Simultaneous degeneration of the supraoptic nucleus, which causes diabetes insipidus, seems to increase the degree of obesity [135]. In monkeys too, damage to portions of the hypothalamus can produce obesity and, at the same time, diabetes insipidus and hypogenitalism may develop. These changes are not correlated with the degree of obesity and are parallel sequelae of the experimental lesions [136]. In all cases, as expected, the obese animals eat more than control animals. Hetherington and Ranson were able to produce exact, reproduceable lesions in the midbrain of the rat by means of a special stereotactic instrument [137]. Large symmetrical lesions of the hypothalamus were regularly followed by obesity, while pituitary lesions or lesions of the connecting tracts to the pituitary had no effect on the weight of the animals. Large laterally-placed lesions of the thalamus produced anorexia [139].

The energy turnover of rats with such hypothalamic lesions is of special interest. Various authors showed that the animals began to eat greedily shortly after the production of the lesions, eating two or three times as much as the controls [140]. There were no changes of gas metabolism. The spontaneous activity of the animals was reduced. Thus, the caloric balance of the animals is disturbed in both senses: There is both reduced output and markedly increased intake. In a few cases, fat is deposited without increased intake of food, merely by reduction of activity. The interrelationship between the disturbance in the hypothalamus and demand for food and reduction in spontaneous activity is unknown.

In human beings, lesions of the midbrain, especially craniopharyngioma ("Erdheim's tumor") are often associated with obesity. The obesity seen in true adiposohypogenital dystrophy is also hypothalamic in origin. If the diagnosis of hypothalamic obesity is being considered, signs of an organic midbrain lesion must also be present; so-called midbrain tests (water, salt, and glucose tests) do not suffice for this purpose.

The obesity seen in Laurence-Moon-Bardet-Biedl syndrome [141] is generally considered cerebral in origin, but it is probably hypothalamic. A number of authors have noted various types of lesions in the floor of the third ventricle in these cases. Thannhauser observed a patient in whom the appetite was so great that he stole anything which was edible (Figure 14). Other signs of this disorder are retinitis pigmentosa or atypical retinitides, together with various secondary disturbances of vision, polydactyly, idiocy, hypogenitalism, and extrapyramidal signs. Symptomatic cerebral obesity may also occur in cases of internal hydrocephalus, especially if associated with small tumors of the fourth ventricle. It is likely that the increased pressure in these cases causes flattening of the floor of the third ventricle, producing lesions which cause the increased appetite. Cerebral obesity is thus identical with hypothalamic obesity.

The evaluation of constitutional or genetic factors is difficult. Anthropological studies do not favor an assumption that certain body types predispose to obesity. The term "lipophilia" has been suggested by von Bergmann to suggest a constitutional tendency of the body to deposit fat, but there is no experimental confirmation of this concept. It is not known if obesity can be inherited. Environmental factors are difficult

to exclude in studies of human obesity, and Verschuer's observation that identical twins show a greater discrepancy in body weight than in other anthropological characteristics probably is a reflection of the great importance of environmental factors [142]. Danforth, on the other hand, showed that in a certain strain of wild mice there is a gene which regularly produces obesity as the result of increased ingestion of food [143]. Other obese strains have recently been reported.

Psychogenic types of obesity include those due to habitual overeating, poor resistance to the offered food, primary love of eating, and substitution of eating for other desires which are not satisfied. Habitual overeaters include elderly people, in whom the caloric requirements go down while the caloric intake remains unchanged or even decreases somewhat. Bed-ridden patients and other patients limited in their ability to move belong to this group, as well as former patients with hyperinsulinism following successful surgery (p. 60). The fundamental finding in these patients is that the intake of food, as compared with the previous requirements of the patients, is not increased. This is not the case in the second group of psychogenic obesity patients, in whom the intake of food is excessive, usually because of custom or merely excessive contact with food. In this group belong fat children, butchers, cooks, beer-drinkers, etc. Such patients are often difficult to distinguish from those of the third group, the gourmets who overeat. This is the Falstaff type of individual who enjoys all the joys of life, including eating. Such patients are the pleasant, jolly fat individuals, although psychoanalysis may reveal an entirely opposite picture. In contrast to these are those people who use food as a substitute for the satisfaction of other appetites which are no longer attainable. These are the patients who become fat during the menopause.



Fig. 14. Laurence-Moon-Bardet-Biedl syndrome in a 14 year old boy. (Patient of Dr. S. J. Thannhauser.)

with age, and on widowhood, and people living in institutions, for whom eating and sleeping afford the only joys remaining in life. Even if it is impossible to categorize a particular patient exactly, classification should be attempted for obvious prognostic reasons.

It was formerly customary to distinguish a number of endocrine types of obesity (pancreatogenic, pituitary, genital, thyrogenic), but it has been shown that the endocrine organs are not diseased in these cases. This finding led to a complete negation of any endocrine influence on the development of obesity. E. H. Rynearson has insisted that the only glands which play a role in the development of obesity are the salivary glands. However, the endocrine glands are concerned with the distribution of fat so that, as a sequel of certain endocrine disorders, there is a typical localization of the deposits of fat although the actual deposition of fat itself is not of endocrine origin.

The distribution of fat is characteristically different in the male as opposed to the female. In the man, fat is deposited primarily at the trunk, especially at the abdomen, the back, and the nape of the neck. The extremities and the buttocks remain free of fat (Fig. 15). In the woman, on the other hand, more of the fat is deposited at the flanks, the upper arms, the thighs, and the buttocks (Fig. 16). The thick layer of subcutaneous fat even in the female of normal weight is responsible for the fact that the contours become soft and rounded. There is also a "childhood type" of fat distribution in which the fat is deposited at the abdomen, the hips, the thighs, and the up-



Fig. 15. Distribution of fat in the typical obesity of the male.



Fig. 16. Distribution of fat in the typical obesity of the female.

per arms. The joints of the hands and ankles are usually slender, while the backs of the hands and feet become fat. With special attention to the slender joints, the childhood form of obesity was previously called "pituitary" since childhood obesity was usually thought to be of pituitary origin. When obesity becomes too great, these sites of predilection no longer are capable of storing all the fat, and intermediate types of distribution follow. Such an intermediate type is also found when sexual differentiation partially diminishes following the menopause; then, in the woman, in addition to the typical fat distribution, increased amounts of fat appear at the abdomen, while the extremities again become thin.

Postmenopausal obesity is often classified as endocrine and explained as due to absence of ovarian activity. This concept is incorrect. There is no such thing as obesity due to genital (i. e., hypogenital) causes. A basic requirement for the endocrine nature of a disorder is the production of the disorder following extirpation of the organ involved and improvement of the disorder by substitution therapy. Neither oophorectomy nor menopause regularly produces obesity. If obesity occurs because of the psychogenic changes which accompany cessation of ovarian function, hormone therapy is successful only if the psychic changes also respond to treatment. Male hypogenitalism also does not cause obesity. Different animals react differently to castration. Birds show an increase in weight; sheep and goats show slower growth and do not become obese; rats show no changes with respect to their fat content. Castration of men may result in either fat or lean eunuchs, independent of the time of operation. Here too psychic factors are of predominant importance.

The diagnosis of Fröhlich's disease (pituitary obesity with true hypogenitalism) is made too frequently. As a rule, these cases are due to overeating, and the type of fat distribution is that seen in childhood, incorrectly termed "pituitary." In these young fat people, puberty often is delayed, probably as a result of the obesity itself. However, there is no indication for hormonal therapy. Cryptorchidism, if present, should be corrected. As a rule, as development proceeds, the body weight becomes normal and puberty occurs of itself. If the physiological thinning of fat children at puberty is overly delayed, an appropriate diet can induce loss of weight, and then puberty follows. Occasionally, adults are seen who have become obese and at the same time have lost their potency and libido; such cases also respond better to diet than to hormones. It is questionable if the pituitary gland has any direct influence in causing excessive deposition of fat in adiposo-hypogenital dystrophy. There are no experimental data for the concept that any of the isolated pituitary hormones produce obesity. Anatomically, Fröhlich's own patient showed a tumor of the hypothalamus, and the obesity of this patient should be thought of as due to a disturbance of hypothalamic regulation rather than as a pituitary abnormality.

In hyperfunction of the *adrenal cortex* obesity is also not the rule. Some of the patients with Cushing's syndrome are obese, especially when the disease begins in children, but the weight may be normal in others and may be low in advanced cases. Even the patients with subnormal weight show the typical distribution of fat at the face and neck (buffalo hump). cursory examination may thus incorrectly suggest the presence of obesity. Occasionally the basal metabolic rate in Cushing's disease is low. More important in the development of obesity is the excessive gluconeogenesis from protein [144]. Other authors attribute the increase in weight, which may also occur after the use of cortisone, to the general feeling of well-being of these patients.

Reduced appetite is often a protective device against obesity in hypothyroid patients. The danger of storing energy is especially marked when the basal metabolism

is low and the energy production corresponding low. In most patients with myxedema, deposition of fat is merely simulated by the retention of fluid, and patients with severe myxedema do not become fat.

The relationship between deposition of fat and hyperinsulinism is of special interest. Patients with this disease quickly learn to prevent attacks by small carbohydrate feedings taken throughout the day. The result, in many cases, is obesity. Patients with functional hypoglycemia do not become fat. This fact argues against the assumption that hypoglycemia reflexly causes excessive hunger, or that the increased production of insulin itself causes deposition of fat. In cases following successful operation, with return of the blood sugar to normal, appetite and obesity may persist. This fact again demonstrates the influence of habit on the genesis of the obesity.

Metabolism in Obesity and Experimental Obesity

The metabolism of obese individuals shows certain deviations from the normal. However, it cannot immediately be inferred whether these changes are causes or results of the obesity. Findings in fat people and in people who are becoming fat are thus of no help in determining the pathogenesis of the obesity. If metabolic changes are the cause of a case of obesity, they must have been present before the obesity and persist after loss of weight. If they are the result of obesity, they must subside as the obesity subsides, although sometimes irreparable damage may have been done. Studies in people of normal weight who have been fat give no clues as to the patho-

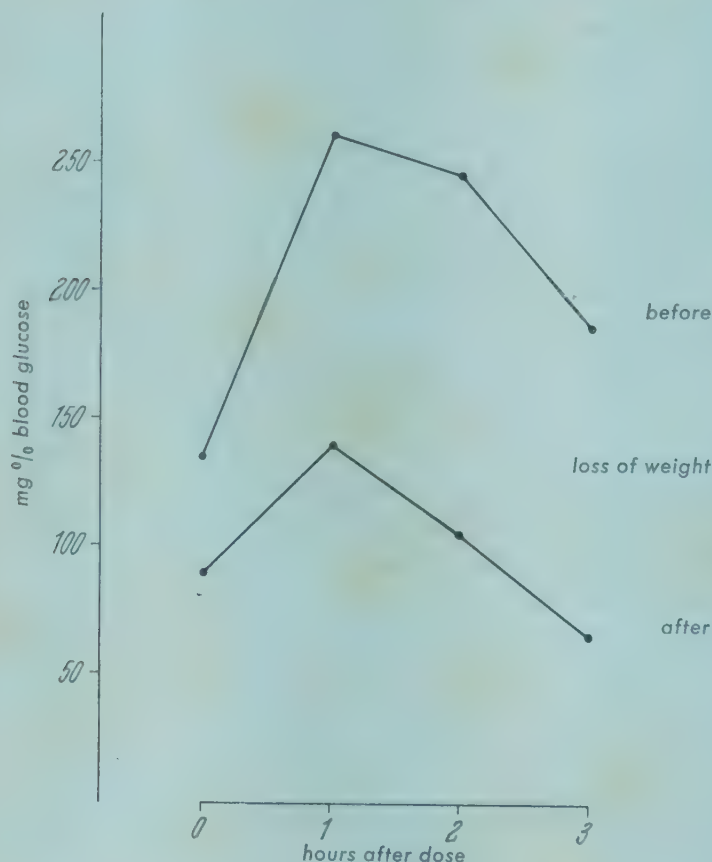


Fig. 17. Glucose tolerance curves in obese patients before and after loss of weight. The dose of glucose was 1.75 grams per kilogram of ideal weight. From Newburgh and Conn [146].

genesis of the obesity; comparative studies in fat people before and after loss of weight show normalization of the metabolism in parallel to the normalization of the body weight. In man, therefore, a pathogenetic metabolic abnormality cannot be found for obesity.

The *protein metabolism* in obesity is normal [145]. The excretion of creatine corresponds to the ideal weight. Patients with Cushing's disease are an exception: in them, negative protein balance may be present.

Obesity can lead to diabetes, which then responds well to dietary management. Newburgh and Conn [146] showed that the abnormal glucose tolerance test present in obese persons becomes normal when the weight returns to normal (Fig. 17). Fructose seems to undergo a similarly disturbed utiliza-

tion [147]. However, it has not been established whether the "diabetic glucose tolerance" curve of obese persons indicates a relative deficiency of insulin.

Fat passes abundantly into intermediary metabolism in obese individuals and the delayed utilization of the hexoses can thus be explained by the fact that, when the amount of fat is increased, fat is oxidized preferentially. According to J. Mayer's glucostatic mechanism [148], a disturbed oxidation of glucose stimulates the appetite center in the central nervous system (e. g., as a result of hypoglycemia or insulin deficiency). Such a disturbance may have been the cause of excessive appetite in one of Mayer's breeds of mice with hereditary obesity. There is no evidence for a glucostatic mechanism in human obesity.

The fat which is deposited as a result of overeating consists almost entirely of triglycerides. The metabolism of depot fat has been considered in the past to be very sluggish, because the total fat content of the body changes only very slowly. However, the theory can no longer be maintained for, as is now known, the fatty acids of fat depots are constantly and rapidly being exchanged [149]. Anatomic, embryologic, and biochemical investigations have confirmed that the process of fat deposition is a dynamic one [150].

The experiments of Strang [151] permit the formation of a fairly complete picture of the metabolism of the obese individual in which the deviations from the normal are considered to be the results, not the cause, of the obesity. The average fasting R. Q. of obese persons is lower than that of normal people. Feeding a mixed test meal containing 40 grams of protein, 26 grams of fat, and 52 grams of carbohydrate – the R. Q. of such a mixture is theoretically 0.83 – results in only a slow rise of the R. Q. Thus, the fat person not only can oxidize his own fat, but seems to burn fat, rather than the other foodstuffs, preferentially. The slight degree of ketosis which is seen in fasting obese persons is also explained by the preferential oxidization of fat, perhaps by the synthesis of adaptive enzymes. A sequel to the increased oxidation of fat is the sparing of the carbohydrate stores (glycogen) and of the body protein. Also, the "entrance" of foods into intermediary metabolism and the depots occurs more slowly, for the demands of intermediary metabolism are largely satisfied by a steady supply of fat and the depots are only partly emptied. The blood carbohydrate level is therefore elevated for a longer time and may simulate diabetes. The endogenous metabolism of protein (the amount used up) is presumably not affected. To the degree that the protein of the diet is utilized, it produces a corresponding specific dynamic action and contributes to the metabolism of carbohydrate or fat according to the types of amino acids within it.

The total daily R. Q. of obese individuals whose weight is constant is the same as the R. Q. of the diet, just as in normal people. When the weight goes up, the R. Q. also goes up because of carbohydrate conversion. When the weight falls, the R. Q. also falls, because of the increased oxidation of fat.

Experimental studies of total gas and caloric balance can be made only in small animals, especially in mice with hereditary obesity and in rats with lesions of the hypothalamus or with obesity following the administration of large doses of gold thioglucose. No peculiarities have been found either in such mice with mendelian dominant hereditary obesity (Danforth) or in rats with hypothalamic lesions. The animals show increased appetite and polyphagia, and reduced spontaneous activity. Digestive functions are normal. The body temperature is maintained in the cold. Fasting animals oxidize primarily fat. The total R. Q. is high during the period of weight gain and becomes normal when the maximum weight is attained.

A different biochemical mechanism of obesity was found by J. Mayer and his coworkers [148] in a different strain of mice with hereditary obesity [152]. This is a recessive trait and is associated with an insulin-resistant hyperglycemia, reduced utilization of oxygen, and reduced body temperature. These mice eat only slightly more than normal controls, but are much more inactive and are very sensitive to the cold. They oxidize glucose and acetate more slowly than do the controls. All these facts indicate impairment of oxidative processes in these animals, presumably in the reactions of the citric acid cycle or in the transfer and oxidation of hydrogen. The obesity which is observed is actually due to a true disturbance of metabolism, even though the precise enzymatic disturbance cannot yet be defined.

Rare Types of Obesity

Regional obesity has been observed only in women (Figure 18). The arms, neck, and upper trunk of these patients remain slim, while the portion of the body below the umbilical level becomes fat. The legs are fat and almost resemble those seen in elephantiasis. Edema of the subcutaneous tissue accompanies the deposition of fat.



Fig. 18. Regional obesity.



Fig. 19. Lipomatous obesity.

A special form of isolated deposition of fat is seen in elderly women and is called "lipomatous obesity." In these cases, lipomata as large as a child's head occur at the buttocks and at the outer and inner aspects of the thighs (Figure 19). Such tumors also occur at the knees. There are transient, intermediate types between this lipomatous

obesity and the flatter deposition of fat as seen in the postmenopausal woman. The lipomatous depots, however, cannot usually be reduced by diet. There is another very rare form of lipomatous obesity which begins between the ages of 20 and 40, affects the arms and legs symmetrically, and is familial in incidence [153 a].

Dercum described "*adipositas dolorosa*" as a special form of deposition of fat [153]. In this condition, which may affect obese as well as non-obese individuals, there occur isolated deposits of fat in the subcutaneous tissue. These deposits are irregular and asymmetrical in form and distribution, and are painful as well as tender to the touch. Generalized weakness and drawing pains in muscles and joints are present. This syndrome occurs chiefly in women, usually after the menopause. The onset is gradual and the course is chronic, without, however, exceeding a certain degree of discomfort. The pathogenesis is thought to be perivascular cellular infiltration with resulting hardening of the fat tissue [154]. *Adipositas dolorosa* of Dercum is a localized disease and must be distinguished from the relapsing febrile nodular non-suppurative panniculitis of Weber-Christian [154 a], which is a generalized disease. Mink and foxes may develop a similar inflammatory disorder of the fatty tissues which is the result of avitaminosis due to the ingestion of spoiled fish.

Clinical Observations on Obesity

The diagnosis of obesity is simple and is made by weighing the patient and noting the thickness of the skin folds. Usually, the patient comes to the physician not because of obesity, but because of some other complaint.

Dyspnea on slight effort is a typical symptom. In young patients, it indicates merely that the oxygen requirement cannot be supplied by the normal-sized heart. Gradually, however, the increased load on the circulation results in disorders of the heart and the blood vessels, especially coronary disease and generalized atherosclerosis. If there are preexisting diseases of the heart or circulatory system, the additional burden of obesity may be especially difficult to overcome. The relationship between obesity and hypertension is still unexplained [155]. Statistical studies have shown a significant correlation between blood pressure and body weight. Reducing diets cause a fall in blood pressure, but only in 50% of the cases is the fall to normal levels. Obesity is probably an aggravating factor in hypertension. In those cases in which the blood pressure returns to normal after loss of weight, the underlying changes in the blood vessels are benign.

Complaints referable to the lungs are rare. The diaphragmatic excursion may be reduced, and bronchitis may occur. Recently, a "Pickwickian syndrome" has been described, consisting of alveolar hypoventilation and drowsiness.

Mild digestive disturbances ("dyspepsia") and bloating are common. Gallstones are frequently seen, and acute pancreatitis may occur. Hernias are not uncommon.

Glycosuria is present in many cases but is not always a sign of true diabetes. In many cases, the glucose tolerance curve, which is abnormal when the patient is fat, becomes normal after he loses weight. If diabetes is present, it persists after the loss of weight, although it is usually milder than before. Diabetes itself is not infrequently associated with obesity, but it is not certain whether it can be considered a sequel of obesity.

Albuminuria and cylindruria may be present. In addition, obese patients tend to have kidney stones, often composed of calcium oxalate. The oxalic acid excreted in the urine is derived chiefly from the ingested food, for the body synthesizes practically

no oxalic acid. A diet rich in oxalate is therefore one of the prerequisites for the formation of such stones.

The organs of locomotion are affected in obesity, so that the patient shows deformities of the feet and may develop arthritis. Back pain is a frequent complaint.

The skin shows varices of the lower extremities and intertriginous dermatitis under the breasts, in the layers of abdominal fat, and in the groin.

Disturbances of potency and libido occur, although they are not very common. They may improve as the obesity improves to the degree that they are sequelae and not an expression of a basic personality disorder which predisposes to obesity. Amenorrhea is often seen and may also disappear after a reducing diet. The delayed puberty seen in fat children comes on spontaneously, often after reduction in weight. Headache and insomnia are frequent complaints.

The prognosis of obesity depends on the age. Childhood obesity is the least dangerous, and in the young adult the mortality of the obese individual is approximately the same as that of the normal person. The situation changes with the 40th year of life, and after the age of 50 the prognosis of obesity is poor, chiefly because of the increased incidence of hypertension and wear and tear. Fisk showed in 1923 that a person aged 45 who is 45 pounds overweight has the same average life expectancy as a person of normal weight with valvular heart disease [156]. Tables 20 and 21 summarize relevant data. Patients whose weight is below the ideal weight have a better life expectancy, and the same results are found in experimental animals [115 c], in which a certain degree of under-nutrition leads to increased life span. The operative risk in obese patients, of course, is greater than in the normal.

Table 20. Effect of Overweight on Mortality in Persons Aged 45 to 50 [157]

Overweight in pounds	Increased mortality above the average for the age (per cent)
10	8
20	18
40	45
60	67
80	116

Table 21. Mortality of certain diseases broken down according to weight. Number of deaths per 100,000 persons in each age group (from L. J. Dublin and H. H. Marks)

	Underweight	Normal weight	Overweight
Organic heart disease	65	80	121
Angina pectoris	14	16	35
Diseases of the arteries	17	23	38
Acute and chronic nephritis	63	82	141
Apoplexy	49	70	110
Carcinoma	62	61	68
Diabetes	9	14	36
Pulmonary tuberculosis	115	57	26
Pneumonia	70	63	59
Accidents	55	60	67
Suicide	27	24	31

Therapy of Obesity

A negative energy balance is without harm. Weight reduction can therefore be accomplished in any patient, even in such conditions as pregnancy, with the sole exception of tuberculosis. Physical activity is helpful if regularly followed, but straining must be avoided, for sports and work may stimulate further eating and thus cause further weight gain.

With the exception of certain rare cases, the therapy must be entirely one of diet. The principle of the diet is that it must be low in caloric content, but not excessively so (e. g., 1,000 to 1,200 Calories), so that it can be varied easily and made acceptable to the patient. The minimum amount of protein must be 60 grams and the rest of the diet can be adapted to the wishes of the patient. Vegetables, which are poor in caloric content but high in satisfying quality, are preferred to sugar- and flour-containing foods. A typical diet, for example, would contain two glasses of skimmed milk or buttermilk, one slice of bread, one egg, two portions of lean meat, four portions of vegetables, one potato, and three servings of fruit (except for grapes and bananas). Butter, oils, and sugar are completely avoided. Umber gives a detailed diet which may be used as a model: in the morning, coffee or tea with a tablespoon of milk and one to two ounces of whole-wheat bread or roll; before noon, 100 grams ($3\frac{1}{2}$ ounces) of fruit; at lunchtime, 200 grams (7 ounces) of broiled meat, 200 grams of vegetables in salt water, 80 grams (3 ounces) of fruit; in the afternoon, coffee with a small amount (one tablespoonful) of milk; for dinner, 100 grams ($3\frac{1}{2}$ ounces) of meat, 100 grams of vegetables, 20 grams of whole wheat bread; and at bedtime, 100 grams of fruit. Instead of these details, simpler instructions may be given to the patient: avoid sugar, potatoes, cakes, noodles, and pancakes; take minimal amounts of fat; use one slice of bread daily; and live mostly on meat, salads, vegetables, and raw or cooked fruits. Water, tea, and coffee without sugar or milk can be taken ad libitum, although the patient must be warned that coffee may stimulate the appetite. Mineral waters often give a feeling of satiety and may be used liberally. Salt can be given in normal amounts except in the presence of edema. Vitamins A and D are given as supplements. There is no special need to add vitamin B. Special diet days, such as days of milk alone or fruit alone, are merely low-calorie days, and may be used once or twice a week as a welcome change from the usual diet. However, a complete dietary system based on such special days must be avoided as potentially dangerous and malnutritious. Low-protein diets have recently been discussed by the Rockefeller group, but in the presence of intercurrent infection they may produce negative nitrogen balance (p. 332).

The patient is instructed to weigh himself at intervals, daily according to some physicians, weekly according to others. He visits the physician every week, then every two weeks and every four weeks. Each visit should include discussion of the dietary problems, the effects of therapy, the patient's outlook, etc. The rate at which weight is lost depends on the caloric requirements and the food intake. A weight loss of 3 to 4.5 pounds per week is excellent in very fat persons, but less is lost as the normal weight is approached (Figure 20). The loss of weight may be masked by a storage of water and may be inapparent until diuresis occurs. Thyroid is sometimes prescribed for its supposed diuretic effect, and mercurial diuretics are employed in stubborn cases.

With the loss of weight, symptoms referable to the obesity subside, with headache subsiding first. The patient generally feels much better, although he is often told that he does not look well. The appearance of skin wrinkles may make him look older,

and the use of massage to improve the skin elasticity is therefore indicated. Occasionally, plastic surgery is necessary in severe cases. Transient loss of some hair may occur as weight is lost. Sequelae of successful weight loss include normalization of the blood pressure and heart, so that digitalization, for example, may no longer be necessary. Glycosuria may disappear and diabetes improve. The prognosis of patients whose weight has returned to normal depends on the duration of the preceding

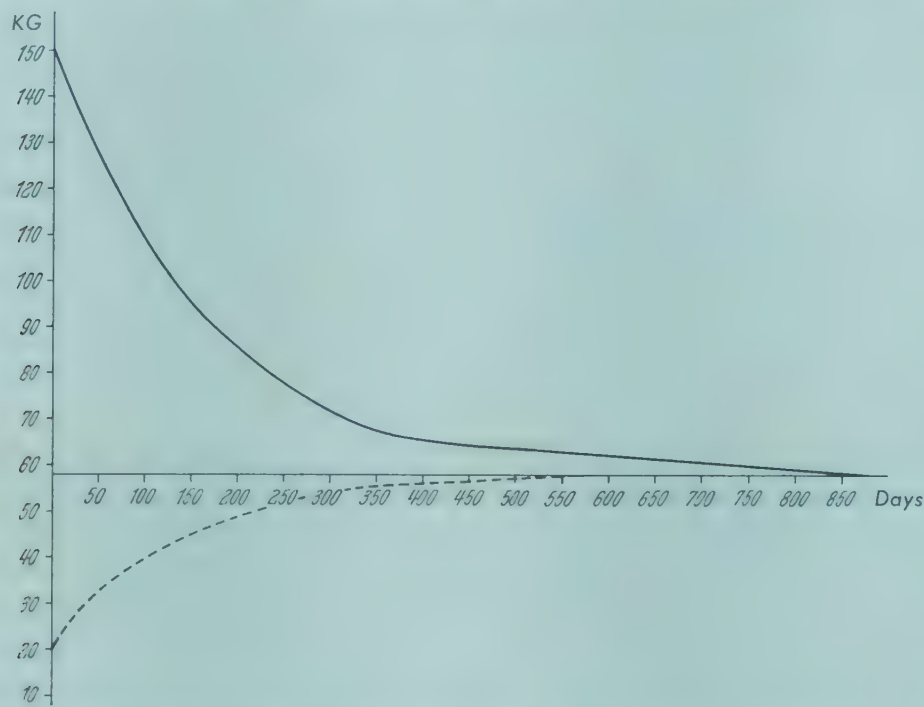


Fig. 20. The body weight under constant conditions of diet, activity, and environmental temperature.

Under these conditions, $\frac{dG}{dt} = Z - k \times G^{2/3}$
where G = body weight, t = time, Z = ingested food. The basal metabolism is $k' \cdot G^{2/3}$. The total metabolism is $k'' \cdot k' \cdot G^{2/3} = k \cdot G^{2/3}$. Solving,

$$\begin{aligned} G &= y^3 \\ \frac{dG}{dt} &= 3 y^2 \frac{dy}{dt} \\ 3 y^2 \frac{dy}{dt} &= Z - k y^2 \\ \int \frac{3 y^2 dy}{Z - k y^2} &= \int dt \end{aligned}$$

The graphic solution is simpler. Let f (caloric value of 1 kg of fatty tissue) = 5,000 cal, let the daily intake = 3,000 cal, and $k = 200$. Then

$$\begin{aligned} f \frac{dG}{dt} \text{ (Cal)} &= Z \text{ (Cal)} - k G^{2/3} \text{ (Cal)} \\ \frac{dG}{dt} &= \frac{Z}{f} - \frac{k}{f} \cdot G^{2/3} = 0.6 - 0.04 G^{2/3} \end{aligned}$$

When $\frac{dG}{dt}$ approaches zero, there is no change in weight in the hypothetical person weighing 58 kg. The value $\frac{dG}{dt}$ can be calculated for each value of G . The figure shows that gain or loss of weight do not show a linear function when the food intake is constant. Analogous curves can be drawn for each type of activity and each diet, permitting the estimation of the probable duration of a cure.

obesity and on the presence of any secondary irreversible damage. Presumably the prognosis returns to normal or almost normal. The patient must be under observation for a long period of time in order to avoid relapse to previous eating habits, and even small gains in weight must be diligently attacked.

Ipecac was formerly used to reduce the appetite, but today the most commonly used drugs are preparations of dextro-amphetamine or related substances. Thyroxin mobilizes tissue water but should only rarely be used because of its effect on general metabolism (palpitation, tremor). Saline laxatives are often necessary for constipation. Massage has no value except for the masseur: the patient's appetite is usually stimulated. The same is true of Turkish baths, which produce weight loss by causing loss of water, but this is quickly replaced at the next meal. Spa therapy is useless and unnecessary.

Undernutrition (Malnutrition)

Definition

By undernutrition is meant the pathological result of an inadequate intake of calories in the diet. In addition to the low body weight, there are characteristic signs which are due to the deficiency of calories. Not all underweight patients are undernourished; i. e., leanness is not the same as undernutrition, although borderline cases may be difficult to categorize. As has already been discussed (Table 21), the life expectancy of thin persons is generally excellent. The prognosis of the chronically undernourished person, on the other hand, is poor. Undernutrition as a result of undereating is not a deficiency disease, such as of vitamins, proteins, or minerals. Such deficiencies may, however, develop in the wake of a low calorie diet. Since the minimum requirement of the adult for essential nutrients is relatively small and is usually supplied even with a small intake of food, even in periods of famine pure forms of undernutrition are commoner than usually assumed. However, it is not always possible to distinguish the effects of avitaminoses, hypoproteinosis, and caloric deficiency from each other. This differentiation is easiest for the avitaminoses (Tables 38, 39), for these conditions often occur even in well-nourished people, and specific therapy is available to eliminate the signs of vitamin deficiency in undernutrition. The signs of protein deficiency and of caloric undernutrition in the adult, on the other hand, are difficult to differentiate, for when the diet contains enough calories the protein minimum is very low (p. 332). In children, and especially in infants, the sequelae of protein deficiency in the presence of adequate calories and adequate vitamins have long been known. The English terms "undernutrition" and "malnutrition" are virtually synonymous, despite the distinction in the German terms for undernutrition ("Magersucht") and deficiency diseases ("Mangelkrankheiten").

Pathogenesis of Undernutrition

Causes of undernutrition include decreased intake of food, disturbances of absorption, and increases in the need for foodstuffs.

The chief cause of reduced intake of food is reduced appetite, a fact which is especially true for the two forms of undernutrition known as anorexia nervosa and Simmond's cachexia. Reduced appetite also occurs frequently following various

organic and psychogenic disorders, such as chronic infections, malignancy, postencephalitic states, and depressions. It should be recalled, however, that in many parts of the world poor diet is the result of poverty and famine, and that these factors also occur periodically in Europe and North America.

Abnormalities of absorption occur in chronic diarrheas of various etiologies, deficient secretion of digestive juices, anacidity, pancreatic disorders, and atrophic disorders of the intestinal mucosa (sprue, etc.). Chronic undernutrition itself may cause disturbed absorption. Poor chewing does not by itself cause disturbances of absorption.

Increased need for foodstuffs occurs during lactation and, to a lesser extent, in pregnancy. The increased need is usually satisfied by the regulation of appetite. When lactating women become thin, there is usually a disturbance of food intake, whether because of an intercurrent organic disease, psychic changes, or poverty. The loss of weight seen in patients with thyrotoxicosis and chronic febrile disorders is likewise due to increased utilization, but it is unclear why the regulation of appetite does not cause an increased intake of food. The undernutrition of a patient with uncontrolled severe diabetes can be explained by the persistent loss of glucose; today, diabetics usually remain thin for therapeutic reasons. The pathogenesis of the cachexia in cancer and in the presence of intestinal parasites is unexplained. As a rule, the basal metabolism (heat formation) and the composition of the stool (absorption) are normal; a toxic action of products of degradation, endogenous pathological substances, or irritant toxins probably result in disturbed appetite. Psychic factors are also of importance in such cases.

Clinical Observations on Undernutrition

Numerous studies of undernutrition have resulted from the last world war [158, 159, 160, 160 a, 160 b, 160 c, 160 d]. The chief symptoms are listlessness, loss of energy, and fatigue. The patients show a typical appearance, with protruding cheekbones, increased intercostal spaces, sharply defined pelvic bones, projecting spinal processes, accented shoulder blades, increased supraclavicular and suprasternal notches. In extreme cases, emaciation is so pronounced that the patient resembles a skeleton. The skin is pale yellow, dry, and cool, and becomes cyanotic in the cold. Spotty brownish pigmentation may be present, and folliculitis may appear at the upper arms and thighs. The skin folds are very thin. In children, growth is arrested. The posture and gait of the patient are characteristic, with the head sunken on the breast, the body and arms hanging forward, and the knees slightly bowed. Undernourished persons have a low body temperature and chill easily. They drink large amounts of fluid and have a large urinary volume. Atrophy of the intestinal glands and diarrhea occur, so that "diarrhea and polyuria are perhaps the most demoralizing aspects of the late stages of malnutrition" [McCance 160 d].

In many cases, the total metabolism is subnormal, with the basal metabolic rate under -30% in clinically severe cases; values as low as -50% have been reported. The body temperature is usually lowered; Leyton found an average value of 96°F [159]. There is no good experimental information with regard to Bürger's findings of a reduction of the enzyme content of the body ("afermentie") [161]. The urine is normal. The plasma proteins are only slightly reduced. Changes in the albumin: globulin ratio are part of the picture of protein deficiency, but, at most, the γ -globulins may be slightly reduced. The blood glucose is reduced. The glucose tolerance curve is flat, but after the double loading procedure of Staub the curve shows the diabetic

type of increased second spike. Some authors state that the oral tolerance curve is flat, but that following intravenous administration of the glucose, it is normal. In any case, the glucose tolerance curve is largely dependent on the severity of the undernutrition [162]. The plasma levels of phospholipids and cholesterol are normal or low, but the neutral fat may be elevated.

The blood pressure is low. Sinus bradycardia as low as 30 may be present during rest, and low voltage and nodal rhythms are seen on the electrocardiogram. The venous and capillary pressures are low. The stroke volume of the heart is small. Because the circulation is sluggish, the patient may develop venous thrombosis in the thighs and the iliac veins, with secondary pulmonary emboli.

The reflexes are markedly diminished but seldom absent. Coordination remains intact. Syncope is frequent, but is of short duration. The autonomic nervous system reacts sluggishly, as shown by diminished reactions to atropine and picrotoxin. The so-called "midbrain tests," such as water loading, salt loading, and glucose tolerance, may be abnormal. Such changes, however, also occur in secondary and psychogenic forms of undernutrition, and are therefore of no pathognomonic significance.

The psychic changes are especially interesting. The mind is usually clear and precision of thinking is unimpaired. However, lassitude and lack of interest are common, including lack of interest in personal hygiene. The sex drive disappears. When famine is the cause of undernutrition, there is an understandable lack of social consideration in the search for food, which may persist after the return of a normal diet.

Disorders due to a deficiency of one of the major foodstuffs are closely related to malnutrition ("dystrophies"). These conditions are usually, in the adult, merely complications of the malnourished state itself, and show certain signs which have already been discussed (pigmentation, edema, abnormal A/G ratio). In children, however, they may occur even though the caloric intake is normal. Deficiency of protein is of greatest importance, and is seen especially in small children who have just been weaned and then placed on a diet of flour cooked in water, with or without added butter and salt ("Mehlnährschaden" of Czerny). Czerny postulated that the basic deficiency was one of animal protein, and the validity of this theory has been established by the success of replacement therapy shown in elaborate experiments by Frontali [163].

The signs of protein deficiency ("hypoproteinosis" of Frontali) include inhibition of growth, edema, trophic changes of the skin including pigmentation, trophic changes of the hair, diarrhea and anorexia, reduction of the enzymatic activity of the duodenal secretions because of pancreatic damage, fatty degeneration of the liver (with cirrhosis in untreated cases), normocytic or macrocytic anemia with normoblastic hyperplasia of the bone marrow, severe hypoproteinemia due largely to diminution of the albumin (but the α - and β -globulins are also reduced, while the γ -globulins are only minimally affected), reduction of the total electrolyte concentration, increase in the extracellular space, and marked apathy which may be interrupted in older children by periods of marked irritability.

In addition to these signs, which are present constantly, certain other findings are present according to the origin of the carbohydrate diet: avitaminoses (such as, for example, pellagra in corn countries and beri-beri in rice countries). In addition, there are also, especially in the tropics, various types of chronic infections which can also influence the clinical picture. Loss of pigment may be seen in colored races, perhaps because of deficiency of vitamin B₆. The term "kwashiorkor" has been recently used for this syndrome.

Special Forms of Undernutrition

Anorexia nervosa, which was first described in 1868 by Sir William Gull, is the result of reduced intake of food because of a disturbed mental state. This disease affects only women and usually begins before the twentieth year of life. Despite the loss of weight, many of the patients are very active and appear able and fit. The signs are those of undernutrition as described above. Amenorrhea occurs early, is observed in all cases, and is the most important endocrine change [164 a]. Constipation, vomiting, and nausea may be present. The course of the disease depends on the degree of malnutrition. In some cases, it is progressive, with intercurrent infection or severe emaciation resulting in death. Usually, however, the prognosis is not so severe and the loss of weight stops after a certain point has been reached. Occasionally, therapy is successful (Fig. 21, 22).

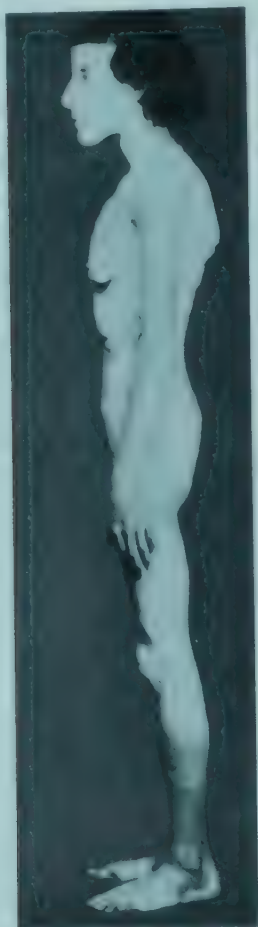


Fig. 21. Anorexia nervosa in a 24-year old woman.



Fig. 22. The same patient as in Fig. 21 after restoration of appetite.

Psychiatrically, too, the disease is quite typical [164]. The poor diet is not always the result of anorexia: the intake of food is reduced for various reasons despite severe hunger and good appetite. Psychoanalysis reveals a personality with poor affect and diminished emotions. There is often a history of overprotective parents, especially the mother, with a strong attachment of the patient for the parents but, at the same time, resentment. There are feelings of inferiority and dependency together with a desire to be independent. Improvement of the psychiatric disturbances with adap-

tation to the surroundings and normalization of the weight occur as often as the opposite chronic course without improvement. Therapy must be primarily psychiatric. It may be necessary to change the situation at home. In severe cases, feeding by tube may be indicated. ACTH and cortisone have a stimulating effect on the appetite and may therefore be useful. Anorexia nervosa must be differentiated from the other psychogenic malnutrition states: the time at which the illness begins, and the presence of amenorrhea, are important differential points.

Panhypopituitarism is the result of a disturbance of the anterior lobe of the pituitary gland. The most important sequelae are myxedema, loss of sexual function and libido, asthenia, and symptoms resembling those of Addison's disease. Only a few cases are severely undernourished (Figure 23). The most common cause of panhypo-

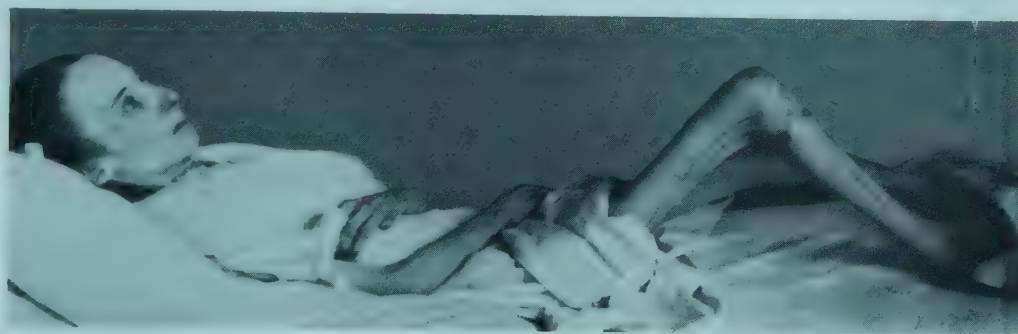


Fig. 23. Pituitary cachexia in a 24-year old woman. Weight 50 lbs.

pituitarism is postpartum necrosis of the hypophysis, seen chiefly after difficult childbirth with severe hemorrhage. Cachexia occurs, if it occurs at all, late in this disease [165], and some authors therefore differentiate true Sheehan's syndrome (hypopituitarism without cachexia) from Simmond's syndrome (hypopituitarism with cachexia).

The pathogenesis of the cachexia is not known. It cannot be produced experimentally by hypophysectomy. The most likely explanation is the occurrence of post-traumatic lesions of the midbrain (Fig. 24) in disease or the development of secondary Addison's disease. The disease begins as myxedema and at first gives the patient a puffy appearance. Amenorrhea, loss of libido, and loss of body hair follow. Signs of diminished function of the adrenal cortex appear later, and Addisonian crises may occur. If cachexia appears, it dominates the picture. The hair of the head becomes thin, the teeth become broken, and the dry ivorylike skin simulates premature senility. The course is progressive. There is neither appetite nor thirst, and cachexia and coma follow. At the beginning, the diagnosis is facilitated by the low basal metabolism, the absence of menstruation, and the blood sugar, but when undernutrition is more severe these signs are less certain, since they are nonspecific and may occur with severe undernutrition due to any cause. The characteristic history and the changes in the hair and skin are helpful in these later stages. Reduced excretion of the corticoids is of questionable significance. Differentiation from anorexia nervosa is possible because of the psychiatric symptoms and earlier age of onset of the latter.

The therapy of hypopituitarism depends on proper diagnosis. Careful replacement of the hormones of the thyroid and sex glands is indicated from the very start. ACTH and cortisone are helpful. Addisonian crises are treated, as usual, with glucose, saline, and desoxycorticosterone acetate. When undernutrition develops, it should be treated

in the usual way as described above. Even in the absence of undernutrition, the prognosis is doubtful.

The *lipodystrophy* originally described by Simons [166] begins with the disappearance of the fat tissue of the upper part of the body, while the fat from the hips down remains normal. This disease occurs only in women and usually begins in childhood. The loss of fat begins in the face and successively involves the neck, the thorax, and the abdomen. The disorder is not particularly progressive, and sometimes only the face may be affected, giving a typical skeleton-like appearance (Fig. 25). Cases with hyperkinetic signs have been described and suggest that lipodystrophy may belong to the group of trophic disorders. As a rule, however, the nervous system is not affected.



Fig. 24. Pituitary cachexia in a 32-year old man. Buried alive in the war. Autopsy showed complete atrophy of the pituitary.



Fig. 25. Progressive lipodystrophy in a 45 year old woman (Simons).

There is no satisfactory therapy. Feeding leads to deposition of fat in parts of the body which were not previously involved. This lipodystrophy of Simons is not related to regional obesity, for in lipodystrophy the loss of fat at the upper parts of the body cannot be altered even when there is marked deposition of fat at the lower parts of the body, while in regional obesity the upper half of the body always shows a normal amount of fat. In addition, regional obesity is never associated with neurological signs.

Neurogenic malnutrition is a trophic disturbance of the fat tissue in diseases of the secondary motor neurons: *i. e.*, atrophy of fat tissue in neurological disorders.

Therapy of Malnutrition

Therapy is not necessarily medical: often, after economic or emotional problems have been resolved, the appetite returns by itself. Psychotherapy may consist merely of good advice or may require painstaking exploration of emotional difficulties in cases of anorexia nervosa. Special high-calorie foods may help stimulate the appetite, and ACTH, cortisone, and thyroxin may have the same effect. In especially severe cases, tubal feeding may be necessary. Sometimes, the appetite returns rapidly after slight weight gain. Usually, however, long-term psychotherapy is necessary, and failures are not uncommon. So-called appetite-stimulating drugs are usually ineffective, but should be tried (vitamin B₁, vitamin B₁₂, tincture of quinine, aperitifs, et al). Insulin is of little use, but DOCA may sometimes be helpful by producing water retention and weight gain, which may wake the patient's interest.

When undernourished patients improve, the natural tendency of the body to store excess energy as fat must be reckoned with. A diet excessively high in calories is not indicated. The synthesis of body protein is stimulated by light exercise, and the patient should stand and walk as soon as possible. Vitamins are indicated to prevent potential vitamin deficiencies. The diet must be high in protein and, at first, should be low in salt content. It is best to give small caloric increases at first and to give five or six meals throughout the day. In no case should the caloric content of the diet exceed that which corresponds to the ideal weight of the patient. Normal weight can be attained in this way (Figure 20). The beginning of a positive caloric balance may be heralded by weight loss due to loss of edema. Complete healing of severe undernutrition may take many months: more rapid weight gain is due only to deposition of fat.

In severe undernutrition, parenteral therapy occasionally becomes necessary. In such patients, the danger of cardiac failure is especially marked and infusions must be given slowly and with caution. Increased pulse rate (a normal pulse rate in malnutrition may be a relative tachycardia), arrhythmias, dyspnea, and increased venous filling are danger signals and demand the use of digitalis. If beri-beri is present because of simultaneous deficiency of vitamin B₁, parenteral administration of the vitamin is indicated for two days prior to parenteral administration of fluids. The use of amino acids is not generally indicated, for they may cause anorexia. An exception is pure hypoproteinoses, in which amino acids or casein are excellent therapy. Blood transfusions and protein infusions are not useful, for the infused protein is broken down only slowly and is thus not readily available for cellular nutrition. The parenteral use of fat is still in the investigative stage [166 a]. The reactions of the malnourished individual to atropine, pilocarpine, and epinephrine are sluggish and occur only after the use of larger doses than normal. The response to insulin is not uniform: both marked hypoglycemia and insulin resistance are seen.

Some patients with essential hypertension seem to be improved by reducing diets. In such cases, underweight is permitted to persist despite a certain amount of discomfort on the part of the patient. If weight must be regained, it must be at a very slow rate. If weight is regained carelessly, severe increase of the hypertension may occur.

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CHAPTER II.

Intermediate Metabolism

By Benno Hess

Biological Oxidation

In 1927, in the first edition of this book, Thannhauser wrote [11]: "Plants have the ability to produce materials containing large amounts of potential energy from the chemically stable system of carbonic acid and water by the action of light. Animals derive their energy from the potential energy of these high-molecular compounds by reconvertng them to carbon dioxide and water. This system of high-molecular compounds, despite their large potential energy, is chemically very stable. Were it not for this fact, their energy would be lost without becoming available to the body. Molecular oxygen, the oxygen of air, is unable to enter into reactions with glucose, amino acids, or fatty acids at physiological temperatures. The oxygen reactions which occur within the body can be simulated in vitro only at very high temperatures. Within the body, however, cellular metabolism goes on at low temperatures. The internal conditions must, therefore, depend on special functions of cellular metabolism. These functions are carried out by catalysts and enzymes . . . it is certain that oxidative catabolism is a chain reaction dependent upon the action of specific enzymes . . .

. . . With regard to pathological events in cellular metabolism, we know of no disease syndrome which might be the result of a pathological type of energy of oxidation. Such a disturbance of oxidative energy has been described by the French as '*ralentissement de la nutrition*,' in which there is reduction of the ability of the organism to cause oxidative degradation of foodstuffs [12]. Actually, clinical and experimental observations of various authors make this hypothesis of reduced oxidations as a cause of disease improbable."

Today, as Wieland [13] predicted, we know that the nicotinamide of the pyridine enzymes withdraws electrons from metabolites by oxidation. These electrons are transferred in the so-called "respiratory chain" from nicotinamide to the alloxazine of the yellow enzymes, from these to the cytochromes, and finally to the iron of the oxygen-transferring respiratory enzyme of Warburg. Here they react with molecular oxygen, which intracellularly reacts only with the ferrous iron of Warburg's enzyme. In this process of electron transfer from substrate to oxygen, the potential energy of the chemicals which are oxidized is set free and is transformed by phosphorylation into other chemical forms of energy by a process known as "oxidative phosphorylation." The overall energy-producing oxidative process is commonly called cellular respiration, and requires molecular oxygen to accept the electrons.

A second chain of reactions which liberates energy is glycolysis, which can readily supply the cells with energy for a short time but does not require oxygen. Cellular

respiration and glycolysis are the basic reactions which supply energy for the constantly changing processes of metabolism. They permit the occurrence of the energy-utilizing reactions by which biochemical or physiological work is carried out. There are other reactions through which energy is set free without being utilized, but these are quantitatively unimportant. Such reactions include the hydrolysis of fats, proteins, and polysaccharides – i. e., the antecedent reactions which produce the actual chemicals to be oxidized in cellular respiration.

In 1927, diseases due to “a disorder of energy of oxidation” were not known. Today, it is known that the manifestations of vitamin B deficiency and of hyperthyroidism and hypothyroidism are the results of damage to the oxidative processes within the cell. A disturbance of cellular respiration is also thought by many authors to be involved in the development of neoplastic cells.

Mechanism of Biological Oxidation

The number of chemical substances which can be used by the body for oxidation is large. Meyerhof [14] therefore postulated that the energy of food is converted into a universal form of chemical energy which can be utilized by all cells for all cellular functions. The mechanism of this conversion consists of degradating (mostly hydrolytic) and then energy-releasing (oxidative) reactions. As Lipmann [15] and Kalckar [16] have shown, the universal form of chemical energy in the cells is the “*high-energy phosphate*” compound containing an energy-rich phosphate bond.

The mechanisms of the biochemical transformation and utilization of energy rest on two principles, the principle of *energetical coupling* of biochemical reactions, and the principle of standard quantities. The former principle was first demonstrated by Meyerhof [14], and states that energy which is released by spontaneously-occurring energy-liberating reactions powers other, energy-utilizing reactions. According to thermodynamic principles, a change of internal energy of a reaction (ΔU) is comprised of the free energy (ΔF) and the entropy of the reaction ($T \Delta S$), where T is the absolute temperature and ΔS is the change in entropy. Thus,

$$\Delta U = \Delta F + T \Delta S.$$

According to the first law of thermodynamics, any form of energy can be converted into any other form. However, the second law limits this generality, for only a portion of the internal energy of a system (ΔU) can be given off as a result of a chemical reaction in the form of chemical, mechanical, or electrical energy for use in work. This portion of energy is designated “maximal work” or “free energy” (ΔF). Only this portion can be used for work and can be taken up by the appropriate systems in the cell. The rest of the energy ($T \Delta S$) consists of heat and is given off as such to the environment. Its amount depends on the temperature (T). (A discussion of entropy is given by Netter [17] and Clark [18]). The energy content of the entropy term can no longer be converted into work and can thus be considered lost. It appears as thermal energy and plays a role, in the body equilibrium, only as regards the regulation of temperature (chemical production of heat, p. 37). When a spontaneous reaction occurs, free energy is liberated, and the reaction is called “exergonic.” When this occurs, the internal energy of the system falls, ΔF has a negative sign, and the reaction supplies energy. The conversion of foodstuffs rich in potential energy to metabolical endproducts is an example of an exergonic reaction. This reaction supplies the energy for life.

A reaction is called "endergonic" when free energy is bound – i. e., ΔF becomes positive. Such reactions never proceed spontaneously. However, if coupled with an exergonic (energy-supplying) reaction, they may themselves take up the energy released by the exergonic reaction and thus proceed to completion. All biochemical reactions which are themselves not spontaneous are coupled in this way, enzymatically, with exergonic reactions either directly or through a transfer system. In the cell, various processes depend on endergonic, energy-utilizing reactions which are bound to exergonic, energy-liberating reactions: the syntheses of proteins, nucleic acids, polysaccharides, and lipids, and the performance of mechanical and electrical work. In a few exceptional cases, heat production can also permit work by a small increase in thermal energy, as in the catabolism of glycogen [19]. This reaction is weakly endergonic and proceeds by virtue of the uptake of thermal energy from the immediate surroundings by heat conduction. In general, however, the heat term of the energy equation plays only a very small role in the body processes.

As Lipmann [15] showed, the body produces a *standard form of chemical energy* – the *energy-rich phosphate bond* – and uses this energy for energy-requiring processes. The high-energy phosphate bond is designated by the symbol $\sim p$. The prototype of compounds containing such phosphate bonds is adenosine triphosphate (ATP), which contains two such bonds. The formation of the energy-rich phosphate bond is the result of a direct coupling of the process of biological oxidation with a reaction through which ATP is formed from inorganic phosphate + adenosine diphosphate (ADP). This reaction results in the formation of a new phosphate bond. When ATP is hydrolyzed, each phosphate bond liberates energy which is given by $\Delta F_0 = -8,000$ to $-16,000$ calories per molecule. The value of ΔF_0 depends on the pH and the temperature.*

ATP can supply the energy requirement of a great variety of endergonic reactions, so that ATP can be looked upon as a general source which transfers energy from energy-producing to energy-utilizing reactions. In the process of energy-transfer, ATP is broken down once more to ADP and inorganic phosphate which again react to re-synthesize ATP. There is thus a "circulation" of the ATP-system of energy-transfer, as Bücher [20] pointed out (Figure 26). Table 22 lists a number of metabolic functions which can derive their energy from the ATP system.

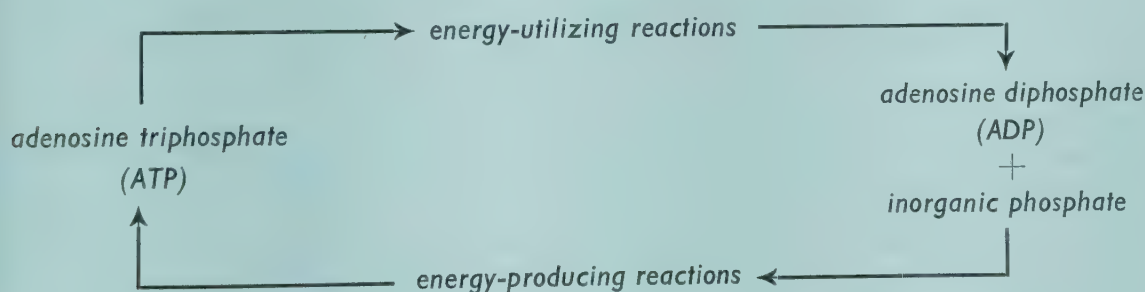


Fig. 26. The ATP system (after Bücher [20]).

There are other phosphoric acid compounds which contain energy-rich phosphate bonds. In contrast to these, there are still other compounds with low-energy phosphate bonds, in which the value of ΔF_0 lies between $-2,000$ and $-4,000$ calories per molecule (Table 23).

* Recent determinations have given $-7,700$ cal/mol at pH 7 and 30°C and $-8,900$ cal/mol at pH 7.5 and 20°C [20 a, b].

In 1952, Stern, Ochoa, and Lynen [21] described another group of energy-rich compounds whose energy of hydrolysis was of the same order of magnitude as that of the phosphate compounds with energy rich bonds. These are the acyl mercaptanes, among which is acetyl-coenzyme A, which plays a central role in the utilization of acetic acid. The free energy of the phosphate compounds can be transferred without loss to the acyl mercaptanes (p. 142).

Table 22. Functions of Various Forms of Energy in the Body

<i>Form of energy</i>	<i>Function</i>	<i>Examples</i>
I. Thermal energy from hydrolysis of ATP; increase of entropy as result of energy producing reactions	Heat production	Temperature regulation, Solubility of lipoproteins, Solubility of fats, Activation of enzymes, Muscle tension, Tendency to infections (?)
II. Chemical energy from the ATP system	Syntheses: phosphorylations, glycoside bonds, C-C bonds, peptide bonds, acetylations, methylations, etc.	Synthesis of highly polymerized substances, proteins, fats, carbohydrates, lipids, nucleic acids, Synthesis of hormones, coenzymes, acetylcholine, Detoxification
III. Other forms of energy	Secretory processes: Electrical processes: Changes in molecular structure:	Formation of hydrochloric acid, Digestion, Functions of the skin, Functions of the kidneys Functions of nerves and muscles Contraction of muscle, Cell mitosis, Phagocytosis, Coagulation (?)

Table 23. Phosphate compounds with low-energy and high-energy phosphate bonds
From [5] and [21]. (See footnote p. 79)

Compound	Type of Bond	ΔF_0 (cal/ molecule)	Temp. (C)	pH
Low-energy				
Glycerophosphate	Ester	— 2,200	38°	8.5
Glyceric acid-3-phosphate	Ester	— 3,000	30°	8.5
Glucose-6-phosphate	Ester	— 3,000	38°	8.5
Fructose-6-phosphate	Ester	— 3,000	30°	8.5
High-energy:				
Adenosine triphosphate	Pyrophosphate	— 10,460	20°	7.8
Adenosine diphosphate	Pyrophosphate	— 10,460		
Pyrophosphate	Pyrophosphate	— 10,460		
Creatine phosphate	Guanidine-phosphate	— 11,800	20°	8.0
1,3-diphosphoryl-D-glyceric acid	Acylphosphate	— 15,000	37°	8.5
Phospho-enol-pyruvic acid	Enolphosphate	— 15,950	25°	6.9
(Acetyl-Coenzyme A	Acyl			
Active sulfate	mercaptane	— 12,000	22°	7.2)
	Phosphosulfate	— 11,000	37°	8.0

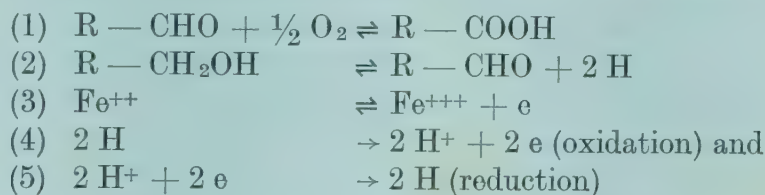
In 1958, Lipmann described a third group of energy-rich compounds, designated "active sulfate," and typified by adenosine diphosphate sulfate (adenosine-3'-phosphate-5'-phosphosulfate) [384].

The total yield of the exergonic oxidation processes can be calculated if the energy-liberating substances are oxidized in a calorimeter and the energy liberated is measured as heat. Thus, for glucose,



giving a total energy of 673 Kcal/molecule (ΔH). The classical studies of Rubner [22], Atwater [23], and Benedict [24] showed that the oxidation of glucose *in vivo* yields the same amount of energy as *in vitro* in the calorimeter. Such measurements of oxidation balance give values for the total energy content of a given foodstuff, but give no indication of the amount of free energy (ΔF) which is actually available for work within the body. The latter calculations can be made by investigating the extent to which the energy of the actual oxidation reactions appear in the form of measurable work. In practice, the calculation can be made *in vitro* by measuring the redox potential, converting the energy set free by an oxidation process into electrical energy and then measuring the electromotive force as electrical work.

Clark [18] and Michaelis [25] showed that, in general, oxidation of a substance can be defined as the removal of electrons from the substance (4), and reduction as the attraction and acceptance of electrons (5). This is true for the various types of oxidation and reduction — oxidation of an aldehyde (addition of oxygen) (1), of an alcohol (loss of hydrogen) (2), of bivalent iron (loss of an electron) (3):



However, loss of an electron occurs only in the presence of a substance capable of taking up an electron — i. e., an "electron-acceptor" or "hydrogen acceptor." Oxidation can thus take place only if there is simultaneous reduction of the acceptor, and vice-versa. There are thus always two systems which react with one another. Reactions are called "redox processes" when there is transfer of an electron between two redox systems, one in the oxidized and one in the reduced form.

The redox potential is a measure of the strength of electron transfer (addition or removal of an electron). A potentiometer is used to measure the electrical work of a redox potential against the potential of another system. When the potentiometer is closed, electrons flow at a certain "pressure" from one redox system to the other. The resulting electrical voltage is a measure of the potential work or the redox potential [26]. A redox system can transfer its electrons only to a system whose potential energy (redox potential) is smaller. Redox potentials are expressed in terms of their voltage against a hydrogen electrode as a reference standard.

The free energy of oxidation can be calculated directly from the electrical work by means of the relationship 1 Faraday (F) = 23,062 calories/volt equivalent. From this ΔF_0 can be derived:

$$\Delta F_0 = -nF\Delta E'_0$$

where $\Delta E'_0$ is the potential difference in volts at pH of 7.0, and n is the number of electrons involved in the oxidation.

Table 24. Redox potentials of some important metabolic systems at pH 7.0
From [5, 41, 67, 26 a]

Substrate System	Active groups	E ₀ '	Temperature °C
<i>α</i> -ketoglutarate/succinate	Lipothiaminpyrophosphate	— 0.600	—
Acetaldehyde/acetic acid		— 0.468	—
—		— 0.42	—
Glucose-6-phosphate/6-phospho- gluconate	reduced pyridine enzyme/ oxidized pyridine enzyme	— 0.44	—
Isocitrate/oxalsuccinate		— 0.332	25
3-phosphoglycerol aldehyde/ 3-phosphoglycerate		— 0.296	35
β-hydroxybutyrate/acetoacetate		— 0.293	38
L(+)-lactate/pyruvate		— 0.204	32
—		— 0.300	—
Succinate/fumarate	—	— 0.026	—
—	reduced flavine enzyme/oxi- dized flavine enzyme	— 0.070	—
—	reduced vitamin K ₁ / oxidized vitamin K ₁	— 0.050	—
—	reduced cytochrome b/ oxidized cytochrome b	— 0.040	30
—	reduced cytochrome c/ oxidized cytochrome c	+ 0.250	—
—	tocopherol quinone reduced/oxidized	+ 0.270	—
—	reduced cytochrome a/ oxidized cytochrome a	— 0.290	—
—	reduced cytochrome a ₃ / oxidized cytochrome a ₃	+ 0.500	—
—	water/oxygen	— 0.740	—

Table 24 gives the redox potentials for some important systems involved in biological oxidations. Considering the potential difference which occurs in the biological oxidation over the total respiratory chain between the pyridine enzyme system and the system water/oxygen, with respect to the free energy change (ΔF_0), one can calculate that the free energy change of an electron pair at 37° equals 53.6 Kcal. If the potential of the pyridine enzymes is -0.32 V and that of oxygen/water is $+0.815$ V, the equation becomes (ignoring the signs)

$$\begin{aligned}\Delta F_0 &= 2 \times 23.06 \frac{\text{Kcal}}{\text{V}} \times (0.32 + 0.815) \text{ V} \times \frac{310}{303} \\ &= 46.12 \times 1.135 \times \frac{310}{303} \text{ Kcal} \\ &= 53.6 \text{ Kcal.}\end{aligned}$$

Thus, in theory, per atom of oxygen used in oxidation, 53.6 Kcal are given off as free energy (ΔF_0) available for work. Comparison with the energy content of energy-

rich phosphate bonds (12,000 cal/molecule) shows that 4.5 molecules of such phosphate compounds are formed at the cost of one atom of oxygen $\frac{(53.6 \text{ Kcal})}{(12.0 \text{ Kcal})} = 4.5$ molecules). In actual practice, only 3 molecules of high-energy phosphate are found per atom of oxygen. The energy yield is thus about 65%, and in actual practice is dependent on many factors, as will be discussed later in this chapter. Table 24 lists the various components of the respiratory chain according to their redox potentials. Each 0.25 V corresponds to approximately one phosphate bond.

The compounds which are responsible for the transfer of electrons in biological oxidations are the *respiratory enzymes*. The active groups of these enzymes have the properties of redox systems. They are distributed in the respiratory chain in accordance with the size of their redox potential, and reversibly transfer the electrons from substrates to oxygen. The active group of the *pyridine enzymes* (p. 106) is *nicotinamide*, which can be oxidized or reduced by taking up or losing electrons, respectively [28]. It is present as a coenzyme in 3 different nucleotide structures within the cell, namely diphosphopyridine nucleotide, triphosphopyridine nucleotide, and coenzyme III (Figure 31). The pyridine coenzymes can be bound by various apo-enzymes. The binding is reversible and relatively easy to dissociate. The constant of dissociation of many of the pyridine enzymes is of the order of 10^{-6} molecules/liter [29] – that is, when the concentration of the coenzyme is 10^{-6} molecules/liter, half of the enzyme is bound to coenzyme. Because of the high dissociation constant, the coenzymes can pass easily from one apo-enzyme to another. A large number of apo-enzymes, which themselves are strictly substrate-specific, therefore links the coenzyme to the reactions with corresponding metabolic substrates. Nicotinamide thus becomes a general electron- or hydrogen-acceptor. None of the active groups of the other respiratory enzymes can react with as many different substances as nicotinamide, none is as strongly dissociated, and none has so many apo-enzymes – hence the importance of nicotinamide in metabolism.

In suitable chemical equilibrium, hydrogen can also be transferred to other substrates by the action of nicotinamide. Nicotinamide is thus important as a hydrogen-transferring agent in fermentation reactions, glycolysis, and fatty acid synthesis and breakdown. At the other end, nicotinamide continually gives up the electrons which it has taken from the substrate to the next member in the respiratory chain – i. e., to the flavine enzymes (flavoproteins), or according to Martius [41] to phyloquinone, which then transfers the electrons by way of the cytochrome chain to oxygen (Fig. 27).

The active group of the *flavine enzymes* (= yellow enzymes) is *alloxazine* (p. 119)[30]. Like nicotinamide, it can bind hydrogen or electrons reversibly. The active group once again is present as a nucleotide, flavin-adenine-dinucleotide or flavin-mononucleotide, which is strongly bound to various specific apo-enzymes. The order of magnitude of the dissociation constant is 10^{-8} to 10^{-9} molecules per liter [29]. In contrast to nicotinamide, therefore, the free nucleotide of the yellow enzyme is virtually non-existent within the cell. Furthermore, it is likely that most of the yellow enzyme is structurally bound within the cell [139]. Because of their specific enzyme protein, the flavine enzymes can react directly, not only with pyridine enzymes, but also with a number of intermediate substances. Such reactions depend on substrates whose redox potentials are less than the potential of the pyridine enzymes (Table 24). The active group of the yellow enzymes is auto-oxidizable; that is, at higher pressures of oxygen it can be oxidized directly by oxygen [30]. It is not known whether this reaction has any special significance in the normal animal. Certainly, it does not have

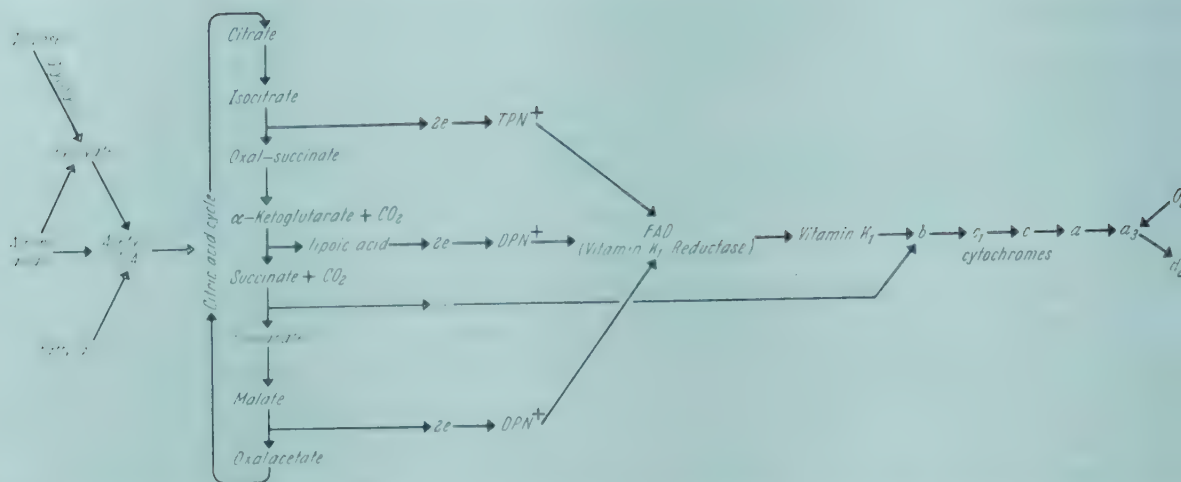


Fig. 27. Schema of respiratory process and its relations to the citric acid cycle (according to Martius [41])

Some authors place Vitamin E between cytochrome b and c_1 . See [56] for details.

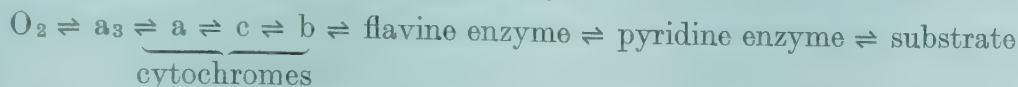
any quantitative importance. The function of the yellow enzymes is to transfer hydrogen from the pyridine enzymes and particular substrates to the heme enzymes of the respiratory chain.

The active group of the heme enzymes (cytochromes) is iron. Iron is bound in a complex manner within the hemes (porphyrins). The hemes are strongly bound to a protein by covalent bonds and therefore cannot be dissociated from their proteins, in contrast to the coenzyme of the pyridine enzymes. In addition, the protein group is strongly anchored within the structure of the cell. The function of iron depends on its ability to change its valence and thus to give off or to accept electrons. One of the heme enzymes, cytochrome b, withdraws electrons from the yellow enzymes. By means of a reversible oxidation-reduction process, the electrons are then successively transferred to three other heme enzymes and finally to a fifth heme enzyme known as Warburg's respiratory enzyme (cytochrome oxidase or cytochrome a_3) [31]. Only this respiratory enzyme of Warburg has the ability to react with molecular oxygen. If one ignores the small role of copper, this complexly bound iron is the only substance present within the cell which can react with molecular oxygen at the concentrations which are present within the body. All other cellular oxidations are carried out without oxygen by the removal of electrons.

The order of the heme enzymes in the respiratory chain can be deduced from their redox potentials (Table 24) or from the time sequence of their oxidation or reduction during alteration of the steady-state equilibrium of the respiratory chain [32]. In this transition, the change in the valence of iron can be followed by recording the changes of its absorption spectrum. Each of the individual cytochromes has characteristic absorption bands, which become prominent after reduction (Figure 28). If one reduces the total respiratory chain of a cell preparation (mitochondria) in the absence of oxygen, maximal reduction results in maximal absorption of all the bands of the cytochromes. If, now, using a special apparatus, one adds oxygen within a very short time (0.002 sec), all the active groups are successively oxidized, as can be seen by the disappearance of all the absorption bands.* The order in which the bands disappear

* The pyridine and flavine enzymes can also be followed by means of their absorption spectra.

indicates how soon after the addition of oxygen the electrons were removed from the individual components of the chain and thus gives their reaction sequence in the respiratory pathway [32] (see also Figure 27):



In recent years, Martius [41] discovered the role of vitamin K₁ in oxidative phosphorylation (see below). He further pointed to the general role of quinones in electron transfer coupled to phosphorylation reactions and also gave some evidence for the function of vitamin E within the respiratory chain. It is apparent that the participation of these compounds in electron transfer has been overlooked because they could not be detected easily by spectroscopy. However, the discovery of a highly active DPN/TPN-vitamin K₁ reductase as flavine enzyme, and certain other data (see below), permit the localization of vitamin K₁ in the respiratory chain between the flavine enzymes and cytochrome b as shown in Figure 27, which also indicates the hypothetical position of vitamin E.

If one considers the overall process of oxidation, it is obvious that only 2 of the components of the chain are constantly used up. These are (1) the *substrate*, from which electrons are withdrawn by the pyridine enzymes; and (2) *oxygen*, which is reduced to H₂O by the electrons which pass along the chain. The members of the chain merely transfer the electrons, oscillating constantly in the process from reduced to oxidized forms and back. When metabolism is in a steady state, they are present partly in reduced and partly in oxidized form.

The speed of utilization of substrate and oxygen is a measure of the magnitude of oxidation. The most important clinical application is in the determination of basal

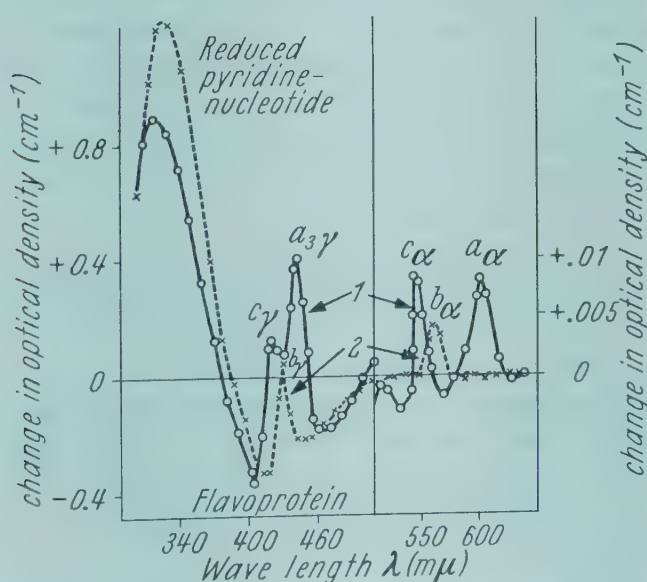


Fig. 28. Difference spectrum of rat liver mitochondria [32]. Curve 1 represents the difference spectrum between reduced and oxidized components of the mitochondria. The right half of the curve has the ordinates at the right; the left half ordinates are at the left. The following bands are shown: reduced pyridine nucleotides, α - and γ -bands of cytochrome c, α -band of cytochrome a, γ -band of cytochrome a₃ (Warburg's respiratory enzyme), the band of flavoproteins. Poisoning of the mitochondria with antimycin A, an antibiotic, produces the α - and β -bands of cytochrome b (curve 2).

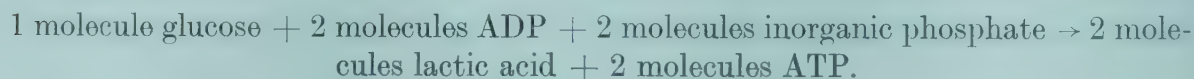
metabolism, which then permits conclusions concerning overall oxidative processes within the body cells. The direct utilization of substrate cannot be measured. In balance studies, this depends on the amount and type of ingested food.

Mechanism of Energetical Coupling

The principle of energetical coupling has already been discussed. According to this principle, high-energy chemical compounds are formed at the cost of potential energy liberated during oxidation. Two processes are known in which oxidation is coupled with phosphorylation (i. e., with the formation of energy-rich phosphate, ATP). The two processes differ from each other with regard to the enzymes involved, the oxygen requirements, and the energy yield [5 a]. One process is *glycolysis*; the other, respiration or *oxidative phosphorylation*. The fundamental differences between the two processes are listed in Table 25.

In 1913, Warburg [33] observed that the respiration of liver cells is firmly anchored within the structure of the cellular granules. However, as Büchner has shown [34], the process of glycolysis can be observed in a water-soluble extract of cells, free of structural components of the cell. If liver cells are destroyed by cytolysis or homogenization, a liver broth is formed which consists of unaltered cell nuclei, cell granules, microsomes and cytoplasm. If these 4 components are separated by fractional centrifugation, oxidative phosphorylation can be detected within the granular structures (the mitochondria of the parenchymatous tissues and the sarcosomes of the muscles), and glycolysis can be detected within the cytoplasm.

Today, it is known that the *glycolytic breakdown* of glucose is catalyzed by 13 protein enzymes and a series of low-molecule compounds [33]. The enzymes can be isolated as pure, crystalline proteins. Of the low-molecule compounds, the ATP-ADP system is the acceptor of the high-energy phosphate; and diphosphopyridine nucleotide is the coenzyme which carries out the transfer of hydrogen. Other essential substances are magnesium ions, potassium ions, 1,6-diphosphoglucose, and 2,3-diphosphoglycerate. The substrates for glycolysis are phosphorylated carbohydrates. If the reagents are placed together in vitro, one can observe the breakdown of glucose to lactic acid as it occurs in the muscles and other cells of the body. The stoichiometric equation for anaerobic glycolysis is as follows:



In 2 steps of the process high-energy phosphate bonds are produced. The transformation of energy-poor phosphate to energy-rich phosphate bonds is linked to oxidation of a phosphorylated substrate, and is known as "substrate-linked phosphorylation." The redox potentials of this process lie between -0.45 V and -0.32 V . In contrast, the redox potential of oxidative phosphorylation lies between -0.32 V and $+0.8 \text{ V}$ (Table 24).

There is only one intermolecular oxidative reaction during glycolysis: the oxidation of D-glycerol-aldehyde-3-phosphate after the addition of inorganic phosphate, with the formation of reduced diphosphopyridine nucleotide. The latter, in the absence of oxygen (when the respiratory chain is reduced), reduces pyruvate to lactate, the chief end product of the glycolytic catabolism of glucose. Stoichiometrically, during glycolysis there is a transfer of only one hydrogen molecule from one substrate to the other, with the formation of two molecules of high-energy phosphate. When glycolysis

proceeds in the presence of oxygen, however, reduced diphosphopyridine nucleotide (DPNH₂) does not reduce pyruvate but causes reduction of the flavine enzyme. The latter then again supplies the electrons, via the cytochromes, to oxygen. Thus, when the oxygen supply of the cells is adequate, no lactate is formed except in the case of malignant cells (see below).

Although glycolysis is well explained, the process of *oxidative phosphorylation* can still only be inferred from balance studies. The actual mechanism of the process is not known. The problem as to how the liberated energy is utilized during respiration has been studied intensively only in the last twenty years. In 1937-1939, Kalkar [35] discovered phosphorylation reactions in the course of cellular respiration. In 1939, Belitzer and Tschibakowa [36] made the first quantitative studies of oxidative phosphorylation in relation to energy transfer. It was not until 1949 that the exact demonstration of the existence and extent of phosphorylation during respiration was made in animals [37] and yeast [38].

When mitochondria are placed into medium containing a suitable substrate (β -hydroxybutyrate, α -ketoglutarate, etc.), inorganic phosphate, ADP, magnesium ions, and oxygen, it can be shown that both oxygen and inorganic phosphate disappear from the reaction mixture. It can also be shown that the inorganic phosphate is converted into compounds containing a high-energy phosphate bond, such as ATP. The ratio between the number of high-energy phosphate bonds formed and the amount of oxygen consumed is relatively constant. Per atom of oxygen, under the usual experimental conditions, some 3 molecules of inorganic phosphate disappear and 3 molecules of high-energy phosphate bonds are formed. The ratio of inorganic phosphate to oxygen (P/O) is therefore 3. The equation of this reaction is



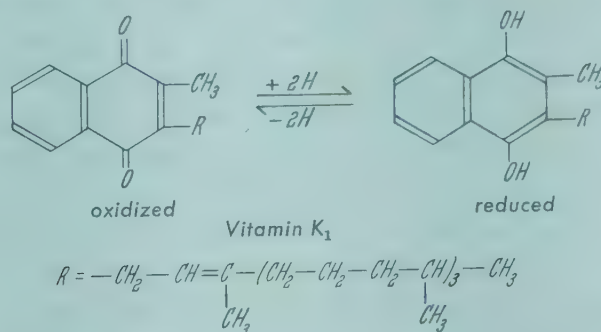
Theoretically, about 4.5 high-energy phosphate bonds could be formed (p. 83). On the average, therefore, some 60%–70% of the energy liberated during respiration is actually converted to high-energy phosphate bonds. The rest of the energy is converted to heat.

The mechanism of the chemical coupling of respiration and phosphorylation is still unknown. According to the redox potential differences, it can be calculated that high-energy phosphate bonds can be produced at 3 sites in the electron transfer process [39]. Various experiments indicate that two phosphorylations occur between the pyridine enzymes and cytochrome c, and one phosphorylation occurs between cytochrome c and Warburg's enzyme. As in glycolysis, the ATP-system is responsible for phosphate transfer in oxidative phosphorylation and ADP is the phosphate acceptor.

Loomis and Lipmann [40] showed in 1948 that dinitrophenol can "uncouple" respiration from phosphorylation; i. e., under the influence of dinitrophenol, respiration continues unaltered or even at an increased rate, but inorganic phosphate is no longer converted to high-energy phosphate. Since this discovery, many other uncoupling substances have been found. Investigations of such substances have increased our knowledge of low-molecular substances involved in oxidative phosphorylation, as well as of endocrine regulation and pathology of the process.

In 1953, Martins and Nitz-Litzow [41] found that Dicumarol also uncouples oxidative phosphorylation. Since there is a structural antagonism between Dicumarol and vitamin K₁ (phyloquinone) in coagulation, they postulated a similar antagonism in the uncoupling action, and assumed that *vitamin K₁* is a *low-molecular member* of the process of *oxidative phosphorylation*. They showed that liver mitochondria of

chickens with vitamin K₁ deficiency form less high-energy phosphate during respiration than normal, and that the P/O ratio is reduced. If vitamin K₁ is added *in vitro* to the liver mitochondria of K₁-deficient chickens, the phosphorylation process is "regenerated" to normal values of high-energy phosphate (i. e., to a normal P/O ratio) in comparison with the control mitochondria obtained from normal chickens. The authors therefore concluded that vitamin K₁ is a component of the respiratory chain



(see fig. 27). Its redox potential at pH 7.0 is -0.6 V. Because of the discovery and crystallization of a DPN-specific phyloquinone-reductase, a flavine enzyme, and because of spectroscopic findings, the authors locate the vitamin K₁ in the respiratory scheme at the site between the flavine enzymes and cytochrome b (fig. 27) [41]. With respect to the physiological significance of this enzyme it is of great interest that the enzyme is inhibited by Dicumarol (50% inhibition in a concentration of $2 \cdot 10^{-7}$ molar Dicumarol) and other anticoagulants. It is possible that vitamin K₁ stores inorganic phosphate directly by forming a phosphorylated intermediate substance, and later, on reduction, supplies high-energy phosphate [41], which then is transferred to ADP.*

Furthermore, Martius gave some experimental evidence that still another low-molecular compound, vitamin E in the form of tocopheryl-quinone, takes part in oxidative phosphorylation [41]. Hummel had previously shown [42] that phosphorylation is inadequate in the muscles of rats with vitamin E deficiency, and that the P/O ratio in these muscles is reduced. Their work has been confirmed by others [41 a]. The redox potential of tocopheryl-quinone is approximately 0.25 V lower than of vitamin K₁ (Table 24).

This difference corresponds to the energy content of one high-energy phosphate bond (12,000 cal/molecule). Tentatively, vitamin E, as its quinone derivative should be located, below vitamin K₁ in the respiratory chain between cytochrome b and c.

Thyroid hormone likewise may participate in oxidative phosphorylation [43], although it is not known whether it is one of the low-molecular compounds which are directly involved in phosphorylation. Its redox potential indicates that it belongs with the cytochromes (see p. 95).

There are marked differences between respiration and glycolysis (Table 25). The cytoplasm undergoing glycolysis contains enzyme protein, low-molecular substances, and a small amount of lipids (about 4%); its nitrogen content is 43.6%. In contrast, the highly organized mitochondrial structures of the cells, which perform oxidative phosphorylation, contain less protein and more lipids (up to 35%, 79% of which is phospholipids.) Electron microscopy reveals that this lipid-rich structure has a double-

* This discovery still leaves unanswered the basic question as to how the role of vitamin K₁ in the clotting mechanism is related to its role in oxidative phosphorylation. The assumption is that ATP synthesized in oxidative phosphorylation links the K₁-sensitive conversion of factor VII to prothrombin [41 b] to the mechanism of oxidative phosphorylation.

lateral outer membrane which surrounds a lamellated lumen. The lamellae (cristae mitochondriales) divide the lumen into many subdivisions. The lumen seems to contain dissolved proteins, nucleotides, and electrolytes such as potassium and sodium. Treatment with supersonic rays separates these substances from the mitochondria (50% of the protein), but phosphorylation is destroyed by such separation. Other mechanical, physical, and chemical methods can rapidly disturb or destroy phosphorylation, which therefore requires an intact structure as a prerequisite.

In glycolysis, the enzymatic reactions are relatively insensitive and occur in solution. Oxidative phosphorylation, in contrast, proceeds, not in solution, but at structures and surfaces – i. e., firm gel-like bodies. It is reasonable to suppose that such reactions are different from reactions which occur in a solution, for the active groups of enzymes are rather rigidly “aligned” within such structures. Such enzymes are “structurally-bound.” It has not been possible as yet to bring such enzymes into solution and still retain their activity.

Simple *balance studies* show that the energy which is set free in both glycolysis and respiration is converted to readily-available energy with the same yield. In both processes, about 65% of the total energy set free occurs in the form of energy-rich phosphate. However, there are marked differences between the two processes when they are compared with regard to the total high-energy phosphate produced per

Table 25. Comparison of Glycolysis (Anaerobic) and Respiration (Aerobic)

	Glycolysis	Respiration
Location	Soluble cytoplasm	Mitochondria
Lipid content (rat liver) in percentage of dry weight	ca. 4.0	34.6
Corresponding phospholipids (%)	—	78.7
Nitrogen content (rat) in percentage of weight of liver	43.6	17.5
Mechanism	Reactions in solutions	Reactions in solid structures
Type of oxidation	Anaerobic: hydrogen transfer	Aerobic: loss of electrons and transfer of electrons to oxygen
Type of phosphorylation	Phosphorylation of substrate	Oxidative phosphorylation
Active groups (enzymes)	Nicotinic acid amide (pyridine-enzymes)	Nicotinic acid amide (pyridine enzymes) Alloxazine (vitamin B ₂) (flavin enzymes) Iron (hemin enzymes)
Low-molecular co-factors	1,6-diphospho-glucose (Leloir ester) 2,3-diphospho-glyceric acid (Greenwald ester)	Vitamin K Vitamin E? Thyroxin? Triiodothyronine?
Electrolytes	K+, Mg++ ions	K+, Mg++ ions
Trace elements		Mn ions
Energy-transferring system	ATP system	ATP system
Substrate	Glucose → Lactic acid	Glucose → CO ₂ + H ₂ O (via citric acid cycle)
Energy yield	2 molecules ATP per molecule of glucose	38 molecules of ATP per molecule of glucose
Sensitivity	Iodoacetic acid Arsenate Fluoride	Tables 32, 33

glucose molecule metabolized. Less than 10% of the total energy of glucose is set free by glycolysis, while 90% is set free by respiration (Table 26). Thus, the energy made available by glycolysis per molecule of glucose is small. In addition, only carbohydrates can supply the substrate for glycolysis, while fats and proteins provide substrates exclusively for respiration via the citric acid cycle.

Table 26. Energy Yield of Glycolytic and Oxidative Degradation of Glucose

		ΔF	High-energy phosphate, number of bonds ($\sim P$)	Yield %	
1 molecule of glucose (from glycogen)	↓ 2 molecules of lactic acid ↓ + [O] 6 molecules CO ₂ + 6 molecules H ₂ O	Glycolysis	— 57 kcal/ molecule	3	65
		Respiration	— 629 kcal/ molecule	38	65
Total		— 686 kcal/ molecule	41	65	

Regulation of Biological Oxidation

Cellular respiration can increase to an extraordinary value, up to 100 times the resting rate. The use of energy by an energy-utilizing reaction induces an increase of oxidative phosphorylation by way of the ATP-system and increased consumption of oxygen. The cellular requirement for energy (ATP) thus controls the level of respiration, which exactly supplies the available energy needed anywhere within the cells. The elements of regulation of biological respiration are the energy-utilizing reactions, the ATP-system, energy-transferring respiration, the ionic milieu, the substrate, and oxygen. The energy-utilizing reactions and the substrate may differ from tissue to tissue. The other functions are almost identical in all the cells of the body.

Various energy-utilizing processes are listed in Table 22. The multiplicity of cellular work implies the presence of a pattern of organization within the body. Not every cell of the highly-developed body contains all possible enzymes: the *cells are*, rather, *specialized*; i. e., specifically oriented to do a particular physiological task. Thus, muscle is specialized to convert energy into mechanical work; nerve, to convert energy into electrical work; liver, to convert energy into work required for biosyntheses, detoxification, etc. In many instances the exact mechanism of energy transformation is quite unknown.

With regard to the substrate too there is a certain specialization from tissue to tissue. The liver oxidizes protein and carbohydrate derivatives as substrates, but not fat; the kidneys use fat. The brain oxidizes carbohydrates almost exclusively; the peripheral nervous system uses fat. Both heart and skeletal muscle preferentially oxidize fat and carbohydrate.

In contrast to these variations, the *reactions which supply energy are the same in all the cells of the body*. There is no difference between biological oxidation within a kidney cell, a brain cell, or any mammalian cell as compared to man. All these cells respire

and phosphorylate in the same way, utilizing oxygen. Experimentally, enzymes isolated from muscle, kidney, and even yeast can be made to take up the energy set free during the respiratory processes of liver cell mitochondria in a combined system.

The ATP system is also the same in all cells. Energy-utilizing reactions need the bonding energy of ATP, and ATP is split in the process into ADP and inorganic phosphate. ADP and inorganic phosphate are then again in position to take up new energy during respiration. If no system requiring energy is operating, the cell is at rest and respiration is at a standstill. Resting mitochondria, despite the addition of oxidizable substances, oxygen, and electrolytes (Mg^{++} and K^{+}), do not begin respiration until the two energy-acceptors, ADP and inorganic phosphate, are supplied (Fig. 26, p. 79). They continue to respire until the inorganic phosphate or the ADP is used up, ATP being formed in the process. When the ATP pool is full, respiration once more rests [127 a]. Thus, respiration is compulsory if ADP and inorganic phosphate are made available through ATP-utilizing processes. The enzymes of oxidative phosphorylation have a great affinity for the ADP and inorganic phosphate present in the cells: K_m for ADP = 2×10^{-5} mol/liter; K_m for inorganic phosphate = $1 \cdot 10^{-4}$ mol/liter [32]. On the other hand, these two substances have less affinity for the corresponding enzymes of glycolysis. There is therefore a preponderance of respiration over glycolysis as long as oxygen and oxidizable substances are present [127 b, c]. The chemical coupling of the ATP-ADP systems to oxidative phosphorylation at the three phosphorylation sites, the high affinity of ADP for respiration, and the kinetic properties of the processes indicate the mechanism of control of respiration. From available data, it is apparent that ADP is the main regulatory substance which controls the respiratory rate and thus determines whether a minimal rate of oxygen uptake at rest or a maximal rate during maximal work of a given tissue occur. There are still other substances which exert a certain degree of control, such as Mg^{++} and Ca^{++} . Their physiological significance is still unknown.

The cellular ATP-pool may be increased in some tissues by direct coupling to another high-energy phosphate pool, the creatine-creatinine phosphate system, which constitutes a reservoir of energy available for quick release, as in skeletal or cardiac muscle (see below).

The proper supply of oxygen and substrates is a prerequisite to respiration. *Oxygen supply to the tissues* is a function of the circulation, of the oxygen-carrying system (hemoglobin), of the lungs, of the autonomic nervous system, and of certain regulatory hormones (epinephrine and norepinephrine). The *supply of substrate* is dependent on coordinating endocrine factors (insulin, pituitary hormones, steroids, glucagon, epinephrine), whose mechanisms of action are not understood as yet. Some *two-thirds of the total oxygen consumption* requires the oxidation of intermediate substances of the *citric acid cycle*. It is this "substrate" cycle which supplies electrons for the respiratory process (Figure 27). The citric acid cycle should, however, be thought of not merely as the source of supply of substrate for energy-producing respiration, but also as a cycle which supplies carbon units for certain syntheses (transamination; synthesis of fatty acids, lipids, and glycogen). Martius discovered [41] and Krebs [45] formulated the process in theoretical detail. There are 8 enzymatic steps, during which keto- and hydroxy-carboxylic acids are catabolized (2 steps each of decarboxylation, hydration, and removal of 4 electron pairs by pyridine- and flavine-enzymes). Keto- and hydroxy-carboxylic acids themselves are formed from carbohydrates, fats, and amino acids and enter the citric acid cycle as keto-carboxylic acids (Figs. 27 and 29). Carbohydrates and fats are introduced by way of acetyl CoA and succinyl CoA, and

amino acids (Table 27) from various intermediate substances. Details are given in textbooks of physiological chemistry.

A small amount of oxygen is used up by dehydration in the degradation reactions which lead to the α -ketocarboxylic acids. This process involves glycolysis, the Knoop-Lynen catabolism of fatty acids (46), and the catabolism of amino acids (p. 138).

The regulation of oxidative phosphorylation is in essence an intracellular interplay between reactions which require and others which produce energy. The ATP-system is fundamental in this interplay. If more substrate or more oxygen becomes necessary, the additional requirement is satisfied from the circulation.

The maximal amount of oxidative phosphorylation depends on the concentrations of the participating enzymes. Their concentration varies from tissue to tissue in accordance with the particular energy requirements. Table 28 gives the maximal oxidative capacity of different tissues expressed as oxygen consumption (measured on the basis of concentration of Warburg's enzyme, cytochrome a_3). In accordance with the different concentrations of the respiratory enzyme, the concentrations of the enzymes of the substrate reactions (e. g., the enzymes of the citric acid cycle, Table 43)

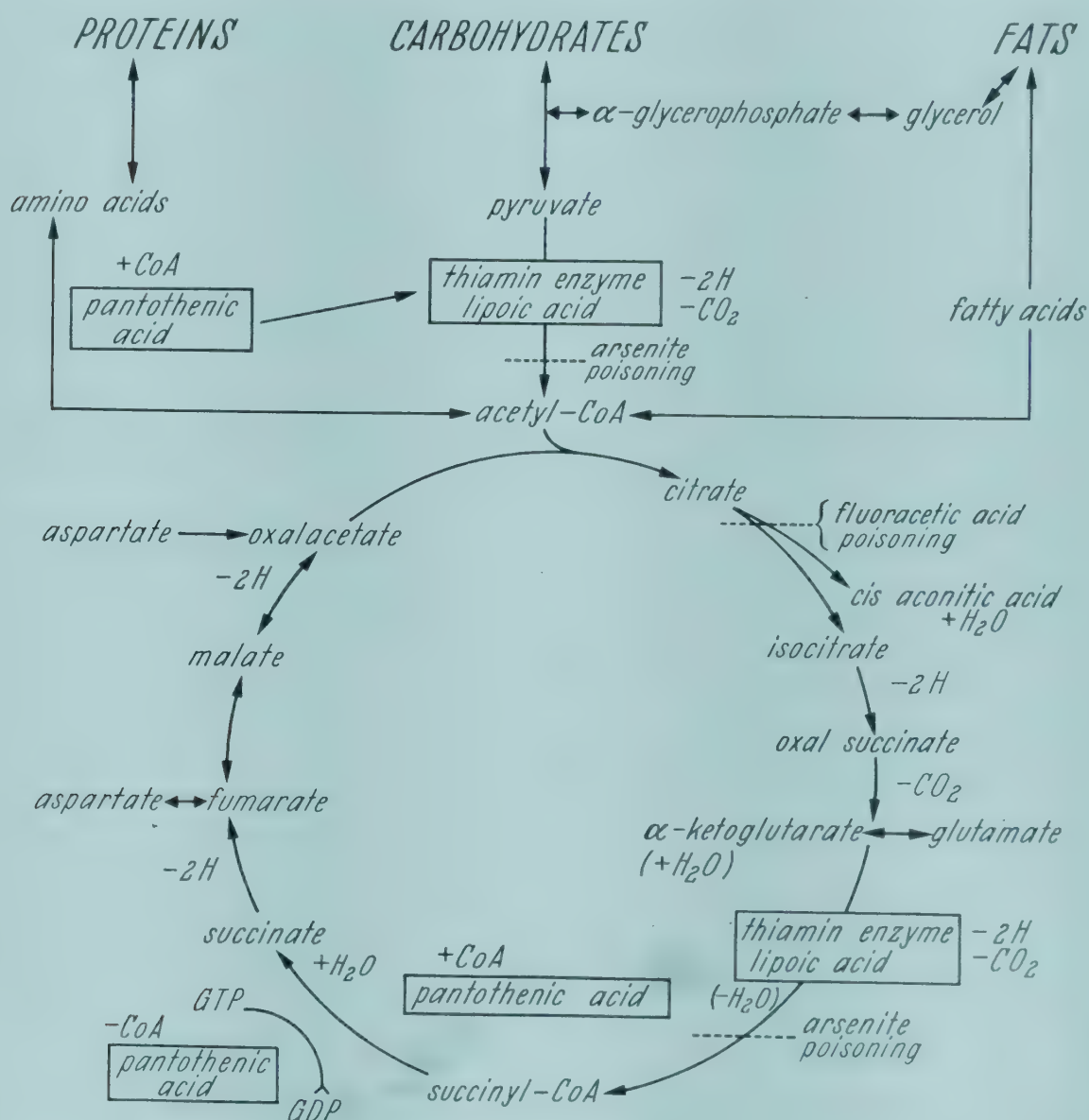


Fig. 29. The Citric Acid Cycle.

Table 27. Intermediate compounds of the citric acid cycle as degradation products of amino acids

Amino acids	Intermediate substance
Glycine Leucine Serine Cysteine Tryptophane Tyrosine Phenylalanine	Acetyl-CoA
Arginine Ornithine Histidine Glutamate Proline	α -ketoglutarate
Tyrosine Phenylalanine Tryptophane	Fumarate
Aspartate	Oxalacetate

and the degree of blood flow to the part ("capillarization") show adaptation to the energy needs of the individual tissues. Adaptation occurs not only to acute but also to chronic increases in energy requirements. This is demonstrated by an adaptation of the components of the total respiratory chain by means of additional synthesis of enzymes (e. g., synthesis of cytochrome C in hyperthyroidism) as well as by increased capillarization.

Consideration of the regulation of cellular respiration in various parts of the body leads to certain important general principles. Cells regulate their metabolism by means of energetical coupling [47]. They do not, however, work only by themselves, but may be set up to perform specialized work as a consequence of their organization into tissues. Wiener calls this "informational coupling;" its elements are the nervous systems, the endocrine secretions, and the circulation. "Informational coupling" is responsible for the coordination of tissue function with other tissue function. No cell is independent. Only the cancer cell is autonomous, at the cost of the normal body cells.

The regulation of cellular respiration must be considered as a mechanism developed in a biotechnical structure of the highly organized level of a living cell. There are multiple pathways of hydrogen transfer from the substrate level within the cell

Table 28. Maximal capacity for oxidation of various tissues. Calculated on the basis of the tissue content of Warburg's respiratory enzyme. After Lang [101]

Tissue	QO ₂ (cu. mm. O ₂ per mg. dry weight per hour)
Cardiac muscle	974
Kidneys	549
Brain	420
Liver	392
Spleen	195
Muscle	180
Lungs	92
Skin	2

structure to the mitochondrial respiration [26 a]. It is further necessary to imply in any complete theory of regulation of respiration the fact that the respiratory, energy-yielding mechanisms are located in the mitochondrial structure, often at some distance from the site of energy-utilizing processes (e. g., muscular contraction) [26 b]. Thus, changes of permeability for chemicals, diffusion times, transit times, spatial conditions, and other factors vary. There is a limited range of variation in the steady state.

The structural morphological requirements for normal processes have their counterparts in the chemical requirements. Thus, pathological conditions may be described from two different points of view, a biochemical and a morphological histological.

Endocrine Regulation of Cellular Respiration

In 1951, Martius and Hess [43] showed that the P/O ratio is influenced by thyroid hormone, and their work was confirmed by other authors [47 a, 47 b]. When thyroid hormone (triiodothyronine or thyroxine) is added to a preparation of liver or kidney mitochondria, or slices of diaphragmatic muscle (rat, hamster, guinea pig, chicken), there is reduced formation of high-energy phosphate in comparison with the amount of oxygen used up, and the ratio of P/O falls. This hormonal effect occurs not only *in vitro*, but also *in vivo*, as can be shown by administering the hormone by injection or by mouth, sacrificing the animal, and determining the P/O ratio in the isolated mitochondria. The yield of high-energy phosphate during oxidative phosphorylation is reduced by thyroid hormone, and the P/O ratio decreases in experimental hyperthyroidism.

The activity of the thyroid glands varies both physiologically and pathologically. The effect of age is especially marked [48, 49]. Comparison of the P/O ratio of liver and diaphragm in the young and the old individual in a given species of animal shows elevation in the old individual, corresponding to increased formation of high energy phosphate [50]. The degree of thyroid activity varies with the seasons, and there are corresponding variations of energy production due to oxidative phosphorylation (liver cell mitochondria) in laboratory animals [43]. If hypothyroidism is produced experimentally by appropriate drugs, the P/O ratio is increased [50].

Magnus-Levy [51] was the first to show that thyroid hormone influences the basal metabolic rate. Basal metabolism, which measures the oxygen utilization of the total resting organism, is composed of the individual oxygen utilization values of each tissue and its cellular components. The largest portion of oxygen is utilized by the cells during oxidative phosphorylation. The energy balance of this process is given by the P/O ratio. Comparison of basal metabolism and P/O ratio shows that there is an inverse relationship: low P/O values occur when the basal metabolism is high, and vice versa. This fact holds both normally and pathologically, and is readily understood. If the energy production (i. e., the P/O ratio) of respiration is reduced, there must be more oxidation than normal to produce the same amount of high energy phosphate. Finally, since the requirement for and therefore the formation of high-energy phosphate remains the same, the only changes which occur are in the utilization of substrate and oxygen, and these are adapted to the altered P/O ratio. Thyroid hormone thus regulates the utilization of oxygen by influencing the P/O ratio, the energy-output due to oxidative phosphorylation.

In experimental hypo- and hyper-thyroidism, the basal metabolism can be calculated approximately from the P/O ratio on the assumption of a constant requirement for high-energy phosphate (i. e., for *readily available energy*). As seen in Table 29, the

Table 29. Comparison of the observed P/O values with the basal metabolic rate*.
From [52]

Exp. No.	Substance	Dose mg/kg body weight	Fall of observed P/O values in % (Diaphragm)	Amount of rise of basal metabolism (%)	
				Observed	Calculated from the P/O fall*
1	L-thyroxin	8.68	— 34%	54	51.5
2	L-thyroxin	3.44	— 44%	71	78.6
3	L-triiodothyronine	11.4	— 26%	41	35
4	L-triiodothyronine	8.4	— 56%	125	127
5	L-triiodothyronine	3.34	— 36%	69	50.6
6	L-triiodothyronine	3.76	— 48%	104	92.4
7	L-triiodothyronine	8.35	— 34%	54	52

* The increase of the basal metabolism (S) is calculated as the sum of a convergent geometric series. The first member of the series (A₁) is the percentual fall of the P/O values: $(S) = (A_1) \frac{1}{1 - \frac{A_1}{100}}$.

In this type of calculation, the P/O ratio for muscle approximates the total metabolism of the body. The percentual fall is determined by comparison with controls.

basal metabolism determined in the living animal can be compared with the P/O ratio found in isolated mitochondria of the same animal, thus allowing comparison of actual and theoretical values.

It has recently been shown that, in vitro, thyroxin and triiodothyronine cause swelling of the mitochondria of rat liver [376], and that mitochondria obtained from hyperthyroid rats show alteration in permeability (loss of potassium, uptake of water) [377]. Histologically, this swelling is the classical cloudy swelling of the hyperthyroid liver, and there is a rise of extracellular potassium corresponding to the loss of potassium from the cells. The swelling phenomena, as measured by turbidity of mitochondria or light scattering effect, reflect the changes in the submicroscopic structure of the particles. It is reasonable to assume that the mechanism of swelling is intimately associated with the chemical action of hormone.

The exact site in the respiratory chain at which the thyroid hormone acts is not known. Its action has been compared with that of other uncoupling substances. Such comparisons, up to now, depend solely upon the P/O ratio: i. e., upon a balance calculation which gives no clue as to the mechanism of action. The thyroid hormone has the properties of a redox system, like the other low-molecule effector substances of oxidative phosphorylation (vitamin K₁ and vitamin E) or ubiquinone. The redox potential of thyroxin is approximately + 0.7 V at pH 7.0.

Under normal conditions the thyroid gland secretes some 100 γ of thyroid hormone per day. When there is an excess of the hormone, the peripheral tissues show reduction of the P/O ratio, and the cells show elevated metabolism. The utilization of oxygen is elevated, the utilization of substrate is elevated, and there is increased production of heat. The clinical picture of hyperthyroidism takes various forms, including classical Graves' disease. The clinical picture of the opposite syndrome, lack of thyroid hormone, is myxedema. Both disorders show disturbed regulation of oxidative phosphorylation (p. 145).

Pasteur's Reaction

Pasteur [53] showed that the fermentation of sugar can replace respiration with regard to energy production: “La fermentation est la vie sans air”. When oxygen is removed from respiring yeast, the yeast begins to show fermentation; when oxygen is added again, respiration again begins and fermentation stops. Warburg [54] called this phenomenon “Pasteur's reaction.” It occurs not only in yeast and other micro-organisms but also in all animal cells; here, however, the end product is not alcohol but lactate. The fermentative process is called “glycolysis.”

Warburg [54] found that all animal tissues split sugar in the absence of oxygen – i.e. glycolysis occurs in every tissue when respiration is limited. Furthermore, glycolysis regularly occurs when oxidative phosphorylation – i. e., the synthesis of high-energy phosphate (ATP) at the cost of respiratory energy – stops. Or, from the point of view of energy, glycolysis occurs when the energy requirement of the cells cannot be supplied by oxidative phosphorylation.

Different tissues show different capacities for glycolysis. Table 30 compares glycolysis and respiration of various tissue cells and also gives the corresponding production of high-energy phosphate. Glycolysis is of quantitative importance only in muscle, the brain, the retina, embryonal tissues, the placenta, and tumor tissue. Even in these tissues, however, the importance of glycolysis is limited, for glycolysis can never replace aerobic respiration for any prolonged period of time.

There is a marked quantitative difference between the ATP-yield from respiration and that from glycolysis. In glycolysis, approximately 19 times as much glucose must be metabolized to give the same amount of ATP as in aerobic respiration, as calculated from P/O ratios:

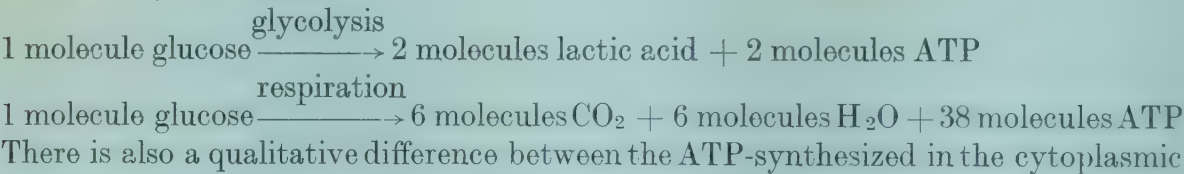


Table 30. Production of energy by various tissues, compiled from Warburg [54], Opitz [55], and Lang [101]

A P/O value of 3.0 is assumed. The Q-values give (1) the oxygen utilization; (2) the corresponding production of ATP under aerobic conditions; (3) the anaerobic production of lactate; (4) the corresponding anaerobic production of ATP in mm³ per mg of dry weight per hour. Column (5) is the sum of the Q values for aerobic plus anaerobic production of ATP (i. e., column 2 plus column 4).

Tissue	Q _{O₂} (1)	Q _{ATP} ^{O₂} (2)	Q _M ^{N₂} (3)	Q _{ATP} ^{N₂} (4)	Q _{ATP} ^{O₂} + Q _{ATP} ^{N₂} (5)	Remarks
Liver	— 15	90	+ 1	1	91	human, in situ. (Dry weight 16.5%)
Kidneys	— 15	90	+ 1	1	91	
Cerebral cortex	— 13	78	+ 2	2	80	
Intestinal mucosa	— 18	108	+ 1	1	109	Maximal!
Skeletal muscle	— 180	1080	+ 200	200	1280	
Retina	— 31	186	+ 88	88	274	
Embryo	— 15	90	+ 25	25	115	
Ascites tumor cells	— 7	42	+ 60	60	102	

Table 31. Comparison of the Energy Production of the Brain during Respiration and Glycolysis, calculated on the assumption of a P/O value of 3.0. (Modified from Opitz [56])

Respiration (Aerobic)			Glycolysis (Anaerobic)			
	$\mu\text{mol O}_2$ per 100 G. fresh brain per minute	$\mu\text{mol ATP}$ per 100 G. fresh brain per minute		$\mu\text{mol lactate}$ per 100 G. fresh brain per minute	$\mu\text{mol ATP}$ per 100 G. fresh brain per minute	Remarks
Man, resting	147	880	Rabbits	1110	1110	15 seconds after de- capitation
Monkey at maximal "effort" (picrotoxin spasm)	289	1730				

space and that formed in the mitochondrial structures. Pasteur [53] had already observed structural degeneration of yeast cells in the absence of oxygen when only fermentation was active. When oxygen is supplied, the yeast again becomes normal in structure and in growth. Calculation of aerobic and anaerobic production of energy in the human brain according to the data of Opitz [55] (Table 31) shows that glycolytic production of ATP can be maintained at the same level as aerobic production of ATP only for 25 seconds. However, it is known that unconsciousness occurs after only 5 to 8 seconds without oxygen, even when enough ATP is present in the cytoplasm. The meaning of these facts is unclear. Some workers believe that ATP formed in the cytoplasm cannot substitute for mitochondrial ATP, but it is just as reasonable to assume that ATP alone is not sufficient for survival and that other substances are also necessary. The first phase of contraction of skeletal muscle occurs glycolytically, after which the muscle utilizes oxygen. Opitz [55] calculated a quotient of glycolysis for skeletal muscle of $Q_M^{N_2} = 200$. This figure exceeds the glycolysis quotients found in the retina and in tumors. Local biotechnical factors (diffusion) may play a role in this preponderance of glycolysis as the primary ATP-producing process against aerobic respiration: The contractile myofibrils lie within the glycolysing cytoplasm, while the respiring sarcosomes, each surrounded by a double-layered membrane, are bound together in the A-bands [56]. Cardiac muscle, despite its high capacity for glycolysis, quickly responds to removal of oxygen with cellular death. Embryonal tissue, despite its high glycolytic capacity, similarly responds to oxygen-lack by malformations [57]. Retinal tissue, in the absence of oxygen, likewise shows rapid death: despite its high rate of glycolysis, after 6 or 7 minutes of oxygen lack, sight is damaged irreversibly [58].

Since glycolysis regularly appears when the cellular requirement for energy cannot be supplied by oxidative phosphorylation, it may be looked upon as an indication of disturbed oxidative phosphorylation. Except in muscular contraction, glycolysis always indicates insufficient oxidative phosphorylation.

Since the discovery of Pasteur's reaction, the search for an explanation of its chemical mechanism has continued. Lynen [59] and Johnson [60] proposed the phosphate theory of the Pasteur reaction, which is based on the different requirement and different affinity of glycolysis and oxidative phosphorylation for the ATP-transfer system. Since both processes require inorganic phosphate and ADP, there is a competition for these substances. The (aerobic) respiration has the greater affinity for

inorganic phosphate and ADP. Hence, in respiration, the stationary concentrations of inorganic phosphate and ADP are reduced to such a degree that the energy-acceptor system is removed from glycolysis and only those minimal amounts of glucose are split glycolytically which suffice as substrate donor for respiration (pyruvic acid \rightarrow citric acid cycle). It is only when oxidative phosphorylation is disturbed that inorganic phosphate and ADP occur free in sufficient amounts to permit the onset of glycolysis. The discovery of uncoupling substances has lent support to this phosphate theory [61, 375]. In uncoupling, the utilization of oxygen increases, but energy is not used for the mitochondrial formation of ATP. Inorganic phosphate and ADP therefore do not react in the mitochondria, despite the high utilization of oxygen, and are available for glycolysis. In such cases, both glycolysis and respiration occur together, and the term "aerobic glycolysis" is used. This can be an indication of uncoupled oxidative phosphorylation. It is not certain whether all instances in which glycolysis and respiration occur at the same time indicate actual uncoupling of oxidative phosphorylation. An important exception, for example, is the glycolysis of malignant growth.

Pathology of Biological Oxidation

Cellular respiration is the most important energy-producing process of life. Disturbances in cellular respiration, as might be expected, have widespread effects on the functions of the cells, the tissues, and the entire organism.

A *deficiency of respiratory enzymes* leads to a reduction of the total metabolism and thus to a reduced production of energy. It may be due to a deficiency of protein, with resulting deficiency of enzyme-proteins which transport the respiratory enzymes. This is especially true when there is a deficiency of the essential amino acids: thus, for example, tryptophane deficiency in the rat liver leads to profound changes in the oxygen uptake [62]. A reduction of basal metabolic rate may be seen in malnutrition (p. 78), although it cannot be stated for certain that the reduced metabolism is the result solely of protein deficiency. A further cause of enzyme deficiency is deficiency of the active groups of the respiratory enzymes. The chief sign of iron deficiency is of course a deficiency of hemoglobin iron, but reduction of cytochrome iron also occurs; it is also possible that certain types of porphyria are related to disturbances of cellular respiration. More important than iron deficiency is a deficiency of certain vitamins (nicotinamide, riboflavin, vitamin K₁, vitamin E), which are the active groups involved in oxidative phosphorylation. Reduction or absence of these vitamins leads to typical disturbances of cellular respiration.

Substrate deficiency can occur as a result of disturbed hormonal regulation of the substrate, deficiency of vitamins, or poisoning. A typical example of substrate deficiency is that induced by excessive insulin dosage. Schmidt and Kety [63] showed that when the blood glucose is reduced to a level of 9 mg/100 ml by the administration of insulin, the oxygen utilization of the brain falls from 3.3 cc of oxygen per 100 grams of brain per minute, to 1.9 cc. The brain thus lacks its essential carbohydrate and cellular respiration falls because of deficiency of substrate. The clinical signs of hypoglycemic shock, loss of consciousness, and convulsions are cerebral in origin. Chronic deficiency of substrate is found in beri-beri, in which there is a deficiency of vitamin B₁, which is the active group of the cocarboxylases (the thiamin enzymes). The cocarboxylases catalyze the conversion of the pyruvate formed from glucose into acetyl-CoA, and also the degradation of α -ketoglutaric acid; in this way they supply

essential intermediate substances of the citric acid cycle. When there is a deficiency of vitamin B₁₂, there is reduction of respiration of the brain as a result of substrate deficiency. Clinically, the result is the development of various types of convulsions and psychoses. When the citric acid cycle is interrupted at the site of α -ketoglutaric acid, those tissues are also affected which can use both fats and carbohydrates, such as the heart. Fluoroacetate [64, 65], from which the lethal compound fluorocitrate is formed, and arsenite, which blocks α -lipoic acid [66, 67], poison the citric acid cycle in a similar way. Here too, the deficiency of substrate is manifested in various tissues, especially in the heart and nervous system, which have a high requirement for energy.

From the point of view of energy, *deficiency of oxygen* is equivalent to deficiency of substrate, although clinically it may be different. The results are the same whether oxygen is lacking because it does not reach the tissues (anemia, hypoxia, anoxia, ischemia), or because the transport or activating mechanism for oxygen (hemoglobin, Warburg's enzyme) is poisoned by carbon monoxide or hydrocyanate. When oxygen is lacking, the electrons accumulate unused along the respiratory chain. Oxidation can no longer take place, and energy cannot be set free. The redox systems within the cells remain reduced. In this way, both pulmonary and circulatory disturbances, as well as poisoning and hemoglobin deficiency, ultimately lead to the same mechanism of cellular suffocation.

The effect of deficiency of electrolytes on biological oxidation is not completely understood. Potassium, magnesium, and perhaps manganese ions are necessary for oxidative phosphorylation [68]. Likewise, potassium and magnesium ions are essential for all transphosphorylations [69].

Damage to oxidative phosphorylation may be due to 2 groups of toxic substances, endogenous or exogenous. The first group inhibits electron passage along the respiratory chain, and includes the classical respiratory poisons described by Warburg [54] (Table 32). The other group inhibits phosphorylation, and has no direct effect on the electron passage. These include the "uncoupling" poisons. In addition, mechanical and physical agents can inhibit phosphorylation (Table 33).

Acute and chronic damage to oxidative phosphorylation can be distinguished. It is a fundamental fact that a deficiency of high-energy compounds always affects oxidative phosphorylation, for this process is concerned with the highly-labile mitochondrial structure whose very status demands energy. Thus, in addition to the uncoupling poisons, deficiencies of oxygen, substrate, enzymes, etc., may result in disturbances of oxidative phosphorylation. In all types of damage, the cells first react with an increase of glycolysis. If the extent of damage exceeds a threshold, death

Table 32. Agents which Inhibit Transport of Electrons [32, 32 a, 41, 54]

Agent	Site of action
Carbon monoxide	Warburg's respiratory enzyme
Hydrocyanic acid	Warburg's respiratory enzyme
Barbiturates	Pyridine-flavine enzyme linkage
Other narcotic agents	?
Carcinogenic carbohydrates	?
X-rays	?
Arsenious acid	?
Hydrogen sulfide	?
Antimycin A	Cytochrome b-c linkage
Dicumarol	Vitamin K ₁ reductase

Table 33. Agents which Inhibit Oxidative Phosphorylation

Mechanical Agents:

Supersonic rays
Sand
Foreign bodies

Physical agents:

Freezing (-3°C.)
Heating ($+45^{\circ}\text{C.}$)
Faradic stimulation
Ultraviolet light
X-rays
Radioactive substances
Hypertonic and hypotonic solutions

Chemical agents:

Hydrogen sulfide, thiourea, thioacetamide, arsenic, iodoacetamide, p-chlormercuri-benzoate
Phenol safranin, methylene blue, thionine, Janus green (vital staining of the mitochondria),
p-phenylene diamine, brilliant cresyl blue
Atabrine, Dicumarol and other vitamin-K antagonists, salicylic acid, Aureomycin, Gramicidin,
guanidine, Phlorhizin, Chlorpromazine
Dinitrophenol, Dibromophenol, Triiodophenol, 3',3,5-Triiodothyronine, 3',3,5-Triiodothyro-
acetic acid, Thyroxin, Bile acids, various detergents, Bilirubin
Benzol, Carbon tetrachloride
Calcium

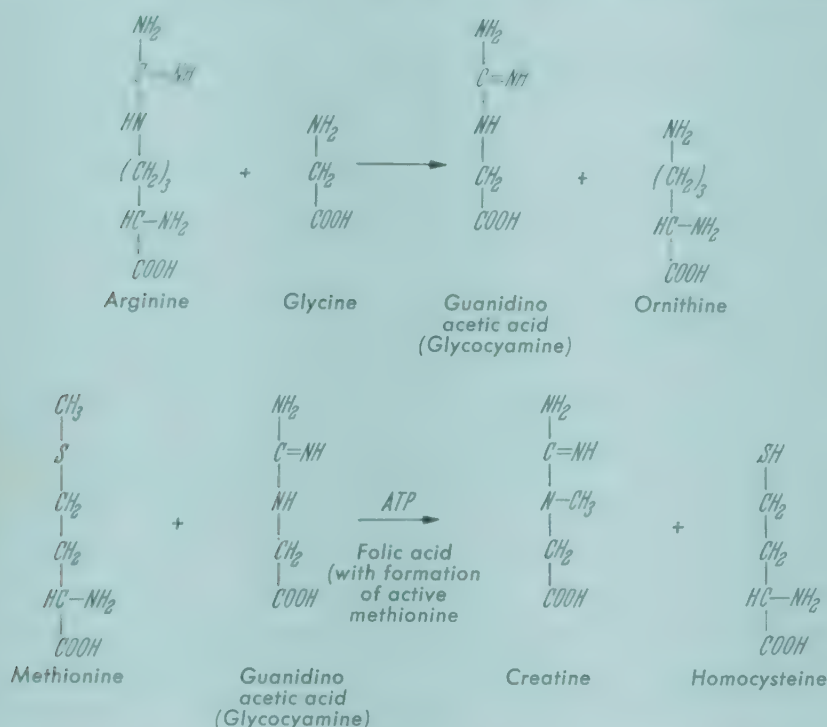
results despite the glycolysis within the cell, with cellular necrosis and the usual anatomic sequelae. If the degree of damage is insufficient to produce death, the cell may become adapted to it. If this occurs, fatty degeneration of the tissues (for example) may occur. Finally, still another result of cellular damage may appear: Warburg [54] wrote in 1927, "Each chronic damage which is not sufficiently great to cause cellular death results in cancer." Today Warburg [54] believes that irreversible damage of the respiration of normal cells is the cause of cancer (p. 105).

Symptoms of Disturbed Cellular Respiration

Disturbances of cellular respiration result in glycolysis and widespread changes in the cellular metabolic balance. The cell excretes the end products of glycolysis, pyruvate and lactate. Small amounts of these substances are oxidized by other tissues, so that the serum levels of these substances are not changed from the normal (pyruvate 400 $\gamma\%$; lactate 5 $\text{mg}\%$). If the extent of cellular damage is too great, the other tissues may not have sufficient capacity to oxidize the acid end products and the serum levels of pyruvate and lactate may rise. At the same time, the pH of the damaged tissues is changed. Both acids also affect the buffer systems of the extracellular space and the blood, so that signs of acidosis may occur. The amounts of pyruvate and lactate excreted by a tissue depend on the ability of the tissue to undergo glycolysis (Table 30). The production of both acids is most noticeable when the cellular respiration of all body tissues is affected by a general pathological condition, as occurs in beri-beri, hyperthyroidism, poisoning by hypnotics and gases, and oxygen deficiency due to anemia or severe pulmonary or cardiac decompensation.

Closely related to the occurrence of glycolysis is the depletion of glycogen of the tissues, especially in the muscles. The glycogen of muscle serves the entire organism as a reserve source of carbohydrate substrate. These reserves are immediately available in emergencies.

Boontano [70] showed that creatinuria parallels glycogen depletion. Creatine is synthesized in two steps by the animal body. In the first step, glycine and arginine undergo transamidation in the kidneys to form glycoxyamine (guanidinoacetic acid) with the liberation of ornithine. Glycoxyamine then undergoes methylation, the methyl donor being methionine (or, alternatively, choline or similar substances). The sulfur-bound methyl group of methionine can be transferred by transmethylation to methyl acceptors. The reaction requires ATP and folic acid as cofactors. An intermediate product of the reaction is active methionine (p. 382), which contains a methyl group bound to the positively charged triple-substituted sulfur (sulfonium) ion:



Creatine is phosphorylated by ATP to give creatine phosphate [71]. This reaction is reversible, and gives rise to ADP. Creatine phosphate belongs to the high-energy compounds ($4 F_0 = -11,800$ cal/mol at pH 8.0). It can react reversibly with ADP to form creatine and ATP, but can also be broken down into creatinine and phosphate (Fig. 30). Creatine is found chiefly in skeletal muscle, also in heart muscle, liver, kidney, testes, brain, and serum. The concentration in the blood is 0.17 to 0.58 mg¹⁰⁰. According to Hobermann, Sims and Peters [72], the total creatine content of the body in an adult of 63 kg, as determined by studies with N¹⁵-labelled creatine, is 115 grams. According to Nevin [73], 44 to 68 percent of muscle creatine is in the form of creatine phosphate. Conway and Hingerty [74] found 24.4 mols of phosphocreatine per kilogram of rat muscle.

Creatinine is present in the normal serum in the amount of 0.4 to 1.65 mg¹⁰⁰, in the urine 1 to 2 grams per day, and in smaller amounts in the tissues. Following intravenous injection, almost 100% is recovered in the urine. After oral administration, some 20% of the dose is lost.

The daily excretion of creatine and creatinine under normal conditions on a meat-free diet shows a constant ratio of the two substances, with the excretion of creatine approximately one-tenth that of creatinine. On a meat-free diet and under basal conditions, the excretion is proportionate to the total creatine and the total muscle mass of the body and varies only within the range of increase and decrease of the total muscle mass [72]. Block and Schönheimer [75] showed by isotope experiments that all the creatinine in the urine ultimately comes from creatine, and that the creatine turnover is equal to the creatinine excretion, is extraordinarily constant, and is independent of ingested creatine. The excretion of creatinine is very constant during bodily exertion, muscular work, and disease, depending on the total muscle mass; the excretion of creatine, however, varies. During growth there is a physiological creatinuria. The female excretes small amounts of creatine constantly, whereas the male does not. The direct production of creatinine from creatine *in vitro* has been demonstrated, but the reaction probably has no biological significance. The production of creatinine from creatine *in vivo* presupposes the formation of creatine phosphate [76, 77]; physiologically, *in vitro*, it can be broken down to inorganic phosphate and creatinine. The mechanism of conversion of creatinine from creatine phosphate (enzymic?) and the significance of the reaction in the cellular metabolism of muscle are not understood. Since the formation of creatinine is independent of body work, it cannot be related to muscular contraction. On the other hand, the formation of creatinine is proportional to the total mass of muscle, which forms approximately 50% of the total body weight.

When oxidative phosphorylation is insufficient for energy requirements, the cells of the body utilize the reserve substances glycogen and creatine phosphate. Creatine phosphate is broken down to creatine, and the free creatine passes through the circulation to be excreted in the urine (Fig. 30). Creatinuria thus is found in many pathological states, all of which have in common localized or generalized disturbances of oxidative phosphorylation. These conditions include deficiencies of vitamin E and thiamin, hyperthyroidism, malnutrition, diabetes, and the effects of certain poisons (Dinitrophenol, urethane, hypnotics, and monobromacetic acid).

Only generalized disorders of cellular respiration are reflected in the basal metabolic rate, and even these can be evaluated only when a true resting state can be attained despite the symptoms of the disease. With the exception of thyroid disorders, reduc-

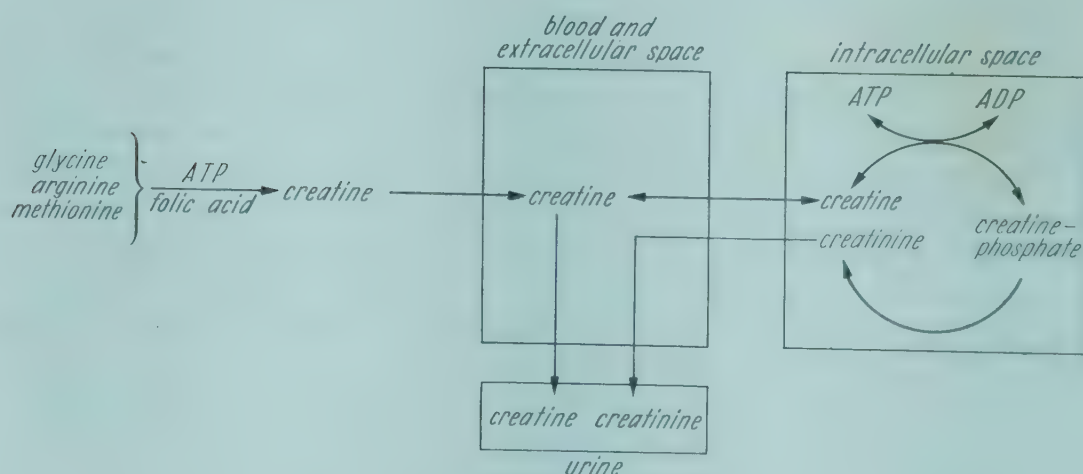


Fig. 30. Schema of creatine metabolism.

tion of basal metabolism is usually a sign of deficiency of substrate or of enzyme or of poisoning of the respiratory chain.

A deficiency of an enzyme in the respiratory chain results in a specific clinical syndrome, no matter which enzyme is affected, for in every case there is insufficiency of cellular respiration and therefore insufficient production of energy. The principal organs affected are the nervous system, the circulatory organs, the skin and mucosae, the bone marrow, and the endocrine glands (especially the adrenal and sex glands). These tissues are known to have a high energy requirement, either because of high rates of regeneration (mucosa and blood cells) or because of other synthetic, electrical, or mechanical functions. There is also clinical and experimental evidence that deficiency of a number of members of the vitamin B complex and of iron can produce glossitis, stomatitis, gastritis, and other alimentary symptoms.

In cases of acute poisoning with carbon monoxide or hydrocyanic acid, which is similar to acute deficiency of oxygen, there is acute blockage of an enzyme which transports electrons. All other disturbances of the enzymes of the respiratory chain develop more slowly, as in deficiency of nicotinamide, riboflavin, other vitamins, iron etc., and the corresponding clinical states are chronic. In this pathological process, regardless of whichever substance is lacking first, the other components of the respiratory chain are affected sooner or later, so that clinically it makes little difference where the first enzyme deficiency is located. In nutritional deficiencies, in addition, there are usually simultaneous deficiencies of several vitamins and nutrient substances. If the deficiency state causes damage to the cells of the gastro-intestinal tract, so that absorption becomes impaired, secondary malabsorptive difficulties may also occur. Thus, a lack of formation of hydrochloric acid in hypovitaminoses may be followed by iron deficiency states (hypochromic anemia). In other avitaminoses, the picture may resemble pernicious anemia. Secondary disturbances of the synthesis or absorption of tetrahydro folic acid, folinic acid, vitamin B₁₂, and intrinsic factor may help explain such events. Similar analogies can be found in considerations of disturbances of the peripheral nervous system and the mucous membranes. The prodromata of all deficiency states are the same: fatigue, digestive disturbances, insomnia, irritability; i. e., nonspecific symptoms. It is only later, when the deficiency disease has become well established, that specific clinical pictures occur. Only at this time can the basic cause for the syndrome be clinically determined.

In addition to the signs of deficiency of enzymes of the respiratory chain, there are signs due to deficiency of substrate and of oxygen, both of which may be acute or chronic. Acute deficiency of substrate is found, for example, following the administration of insulin; chronic substrate deficiency occurs especially in thiamin deficiency and in various types of poisoning (e. g., arsenical). It is probably not possible to differentiate clinically signs of substrate deficiency from those of enzyme deficiency, although it is generally felt that substrate deficiency tends to be associated with disturbances of the peripheral nervous system, the heart, and the circulation. In both types of deficiency, the clinical prodromata are nonspecific, but the definitive clinical syndrome is quite specific (e. g., beri-beri).

The symptomatology of disturbances of regulation found in hypo- and hyperthyroidism has been well documented in the past century, and their pathogenesis is discussed below (p. 145).

Metabolism of Tumors

Tumor cells, like normal cells, require a constant supply of energy for their existence. Warburg discovered in 1923 that the particular characteristic of the energy-producing reactions of tumors is the high proportion of glycolysis [54]. The energy-utilizing reactions of tumor cells include chemical syntheses and transformations of substances which permit their growth. Furthermore, tumor growth is uncontrolled. These reactions are complex and as yet incompletely understood. The fundamental problem of growth is the problem of how form is developed and controlled; this has not yet been solved.

Like all body cells, cancer cells show both energy-producing processes, respiration and glycolysis. In recent years, various workers have measured the rate of each process in purified tumor cell material [78, 79]. Such material includes cancer cells obtained from tissue cultures [79 a] or from ascitic fluid and show all the properties of highly undifferentiated malignant cells when reinoculated into the organism. Ascites tumor cells can be cultured in the peritoneal cavity of mice and can then be isolated almost free from normal cells. Tissue culture cells are grown in chemostats in appropriate artificial media.

All the enzymes found in the respiratory chain of normal body cells are present in ascites cancer cells [80, 80 a]. Determination of the efficiency of oxidative phosphorylation in the mitochondria of ascites tumor cells has given normal P/O values [90 a, 81]. These mitochondria also show good respiratory control [90 a, 80 b] and can be readily uncoupled by various uncoupling agents such as Dicumarol [61].

When Warburg [54] discovered the high level of glycolysis in tumor cells, there was no knowledge of the enzymes and individual reactions involved in glycolysis. Since then these reactions have been studied in detail, and the enzymes involved in glycolysis have been isolated and crystallized. There are no important qualitative differences between the enzyme patterns of normal and cancer cells, as far as has been shown to date. There is also no difference between normal and malignant cells with respect to their substrate cycle (citric acid cycle) and their pentose phosphate cycle.

There are, however, differences between the rates of glycolysis and oxygen utilization in normal and cancer cells. As Warburg discovered [54], the high glycolysis rate of malignant cells exceeds that of normal cells (liver and kidney) by a factor of 60 times and of embryonal cells by a factor of 2.

The respiratory rate under steady state conditions with glucose as substrate has been found by various authors at the lower range of normal [61, 61 a, 61 b]. Consideration of the ratio of the rates of the two glucose-metabolizing processes and of their energy yields gives interesting results. Respiration produces large amounts of free energy from a small amount of substrate (38 to 42 molecules of ATP per molecule of glucose), while glycolysis produces much less energy from the same amount of substrate (2 molecules of ATP per molecule of glucose). Comparison of the rates of glycolysis and respiration with respect to the total energy yield, however, discloses that both normal and malignant cells are able to produce approximately the same amount of free energy in the form of ATP [54]. Table 30 lists data obtained for various tissues, giving the amount of high-energy phosphate (ATP) produced for each Q value, assuming a P/O ratio of 3.0. The ratio of the two processes is shifted in malignant cells towards glycolysis to such a degree that, *in vivo*, glycolysis proceeds in the presence of oxygen ("aerobic glycolysis").

The two types of cells are thus different in that each shows a different predominant type of energy production, respiration in the normal cells and glycolysis in the cancer

cells. The cancer cells thus metabolize much more glucose per unit time than do normal cells. There is evidence that, at least for ascites tumor cells, the predominance of glycolysis in malignant cells has its cause in a potent regulatory mechanism imposed upon the respiratory activity of the intact cell [80 a, 80 b].

The end products of metabolism differ in the tumor cell from those in the normal cell. Respiration produces carbon dioxide and water, while glycolysis results in the formation of lactate and pyruvate. These metabolites are, in part, precursors of the various building units utilized in the growth process. Clinically, it is known that the urine of a patient with diabetes becomes sugar-free when a carcinoma develops, probably because the carcinoma uses up the excess sugar by glycolysis. C. and G. Cori [83] showed increased utilization of glucose and production of lactate in the tumor in a patient with sarcoma of the arm, by comparing the levels of glucose and lactate in the venous blood of the diseased arm with those of the blood in the normal arm. Warburg [54] also showed the production of lactate by tumors by measuring the lactate in the veins flowing to and from the tumors. Lactate impairs the buffer capacity of the tissues, so that acidosis may result.

The energy-utilizing reactions of tumor cells are the reactions responsible for growth. They can be divided on the one hand into syntheses (of proteins, nucleic acids, lipids, polysaccharides), and on the other hand into reactions involved in the physiology of cell division. In general, growth can be orderly or disorderly: embryonal and regenerating cells show orderly growth, while tumor cells show disorderly, uncontrolled growth.

Study of the chemical structure and the synthetic and energy-producing processes of tumor tissues shows that they are qualitatively identical with those of normal cells but that there are quantitative differences with respect to the energy-yielding reactions. Warburg [84] therefore postulated that normal growth is controlled by a mechanism in which the predominance of respiration is of basic importance. When glycolysis is predominant, on the other hand, events take place which may lead to loss of the control of the capacity for growth which is present in each cell as an "ontogenic inheritance." Warburg believes that a cancer cell develops from a normal cell in two stages [54]. In the first stage, the respiration of the normal cell undergoes irreversible damage through the uncoupling action of cancer-producing agents. Thus, certain respiratory poisons are known to be carcinogenic: x-rays, tar, arsenic, mechanical stimuli, urethane, dyes (Tables 32, 33). Chronic oxygen deficiency also damages respiration. Thus, Goldblatt and Cameron [85] found that chronic oxygen deficiency in tissue cultures results in the formation of neoplastic cells, and oxygen deficiency in the embryo has been shown to cause the rapid formation of teratomas [86].

According to Warburg, any single respiratory damage is irreversible. The result of such damage is first an increase in the rate of glycolysis [88] and later a diminution in the respiratory capacity of the cell. If this hypothesis is correct, irreversible damage of the respiratory capacity must be transmitted mitotically to the daughter cells, independent of the cell nucleus; and the mitochondria must be autonomous. Certain recent studies make this concept possible [87].

In the second stage of cancer development according to Warburg, conditions favoring uncontrolled growth increase within the body [54]. The degree of glycolysis increases [88], and there are immunochemical changes which include the development of resistance to the normal cytotoxins of the body. Further studies are necessary to determine the value of this theory.

There are many metabolic interrelationships between tumor and host. The tumor derives from the host many growth factors, electrolytes, glucose, and amino acids.

On the other hand, the tumor excretes the end products of glycolysis into the blood of the host. If the tumor is sufficiently large, these end products, pyruvate and lactate, may be so increased in the blood stream as to produce acidosis. The metabolic events also affect high-molecular substances. Thus, it has long been known that there are changes in the concentration of various enzymes in the tissues and the serum in the presence of a tumor anywhere in the body. The concentration of acid phosphatase in the serum is often elevated in metastatic carcinoma of the prostate. In 1943, Warburg reported an increase of certain glycolytic enzymes in the serum of rats with tumors [33]. The patient with advanced prostatic carcinom also shows marked increase of aldolase [89]. Some patients with cancer show an increase of the serum level of lactate dehydrogenase [90, 371, 373]. Although there are many other examples of metabolic changes, no specific chemical tumor reaction is as yet known.

The Pyridine Enzymes

Physiology of Nicotinamide and Its Enzymes

In 1904, Harden and Young [91] discovered the coenzyme of fermentation, coenzyme I. Warburg and Christian [33] described a similar coenzyme found in studies of the oxidation of glucose-6-phosphate, coenzyme II. They showed that the active group of this coenzyme was a pyridine-derivative, the amide of nicotinic acid, and found that the coenzyme I of Harden and Young has the same active group. Nicotinamide can take up hydrogen by partial hydration and can give up hydrogen again by dehydration. This fact is the basis of its function in metabolic processes.

In 1912, Wieland suggested a theory of biological oxidation according to which the principle of biological oxidation consists in the splitting of hydrogen from the substrates of metabolism. However, free hydrogen is not found in the tissues, so that Wieland postulated the existence of a hydrogen-acceptor. In vitro, he was able to set up experimental systems in which the hydrogen of various oxidizable substrates was withdrawn in the absence of oxygen by artificial hydrogen acceptors (quinone, methylene blue). At first the naturally occurring hydrogen acceptor was unknown, but Warburg and Christian showed that nicotinamide is one such substance. In addition to a hydrogen acceptor, Wieland also postulated a selective enzyme: "The cell is not merely an oven which burns up useless debris." This selective enzyme is known as the apo-enzyme of dehydrogenase. It is specific for a given metabolic substrate. Coenzyme and substrate exchange their hydrogen at the surface of the apo-enzyme. Thunberg and his coworkers [92], using the methylene blue method, have discovered a large number of substrate-specific dehydrogenases.

The apo-enzymes are pure proteins, and many of them have been crystallized (Table 34). Complete analysis of the building stones has thus far been made only for glyceraldehyde-3-phosphate dehydrogenase (Table 35). This compound contains tryptophane, which is also present in other dehydrogenases such as lactate dehydrogenase and α -glycerolphosphate dehydrogenase [94]. When the dietary intake of tryptophane is inadequate, the total oxygen uptake of the body is reduced [62]. Up to now our knowledge concerning the metabolism of the apo-enzymes is relatively poor.

The pyridine coenzymes belong to the class of nucleotides. Three different forms are known (Fig. 31), diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN), and coenzyme III [27]. The first two are dinucleotides, composed of two mononucleotides (nicotinamide mononucleotide and adenosine monophosphate). The binding of the two nucleotides is by means of a pyrophosphate bridge, which is

Table 34. The Crystalline Pyridine Enzymes [3 a, 5]

Name	Approximate Molecular Weight	Turnover number at 38° C.	Co- enzyme	Substrate
Phosphoglyceraldehyde dehydrogenase (= triosephosphatedehydrogenase)	100,000	10,000	DPN ⁺	D-glyceraldehyde-3-phosphate
Lactate dehydrogenase	100,000	73,000	DPN ⁺	L-(+)-lactate
Phosphoglycerol dehydrogenase	100,000	140,000	DPN ⁺	L- α -glycerophosphate
Alcohol dehydrogenase	73,000	17,000	DPN ⁺	Ethyl alcohol, vitamin A
L-glutamate dehydrogenase	1,000,000	?	DPN ⁺ or TPN ⁺	L-glutamate
L- β -hydroxy-acyl-CoA dehydrogenase (= β -ketoreductase)	?	?	DPN ⁺	L- β -hydroxy-acyl-CoA

different from the usual binding in nucleic acids (Chapt. 8). TPN is esterified with an additional molecule of phosphate at the third carbon atom of the adenosine ribose. Coenzyme III is a mononucleotide, nicotinamide-ribose-diphosphate.

The active group of the coenzymes is the N-substituted nicotinamide which, as a pyridine derivative, gives the name to the entire group of enzymes. Nicotinamide is a vitamin (anti-pellagra vitamin or PP-[pellagra-preventing] factor) and belongs to the vitamin B complex. It is synthesized in vivo from tryptophane (Figure 32). Not all highly developed organisms can synthesize nicotinamide in adequate quantities. The human body can synthesize the vitamin in small amounts from tryptophane in

Table 35. The amino acid composition of crystalline glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle. Molecular weight 99,000. From Velick and Ronzoni [93]

Amino Acid	Grams of amino acid per 100 grams enzyme protein
Alanine	6.7
Arginine	5.2
Aspartic acid	12.4
Glutamic acid	6.8
Cysteine	1.1
Glycine	6.0
Histidine	5.0
Isoleucine	9.1
Leucine	6.8
Lysine	9.4
Methionine	2.7
Phenylalanine	5.5
Proline	3.7
Serine	8.5
Threonine	7.6
Tryptophane	2.0
Tyrosine	4.6
Valine	12.0
Amide-NH ₂	1.2

the liver and by bacterial synthesis in the bowel. However, this amount of synthesis is insufficient to supply the nicotinamide requirement of human metabolism so that man requires supplements of nicotinamide in his diet.

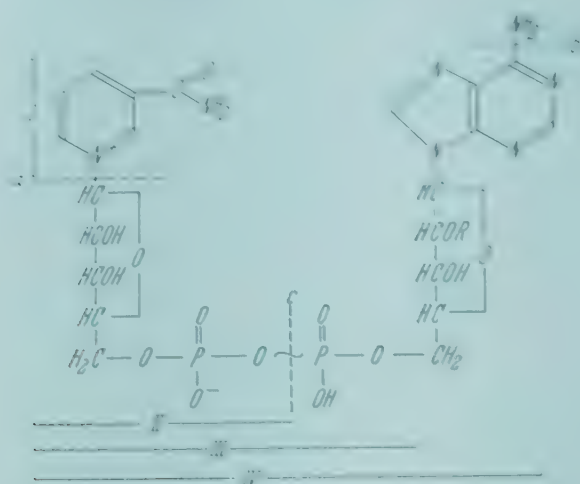


Fig. 31. Structural formula of the pyridine coenzymes.

I. Nicotinamide. – II. Nicotinamide mononucleotide (NMN). – III. Coenzyme III. – IV. R = H: DPN (diphosphopyridine nucleotide, coenzyme I, codehydrogenase I). – R = PO_3H_2 : TPN (triphosphopyridine nucleotide, coenzyme II, codehydrogenase II). – a, b, c Sites of enzymic degradation. See text. ~ P High-energy phosphate bond.

In addition to the pyridine coenzymes and to free nicotinamide, nicotinic acid itself is widely distributed in the animal and plant worlds. Nicotinic acid and nicotinamide are interchangeable as vitamins and the term “niacin” is used for both. Niacin is found in large amounts in yeast, cereals, vegetables, milk, liver, kidneys, and muscle.

Within the body, enzymatic processes convert the vitamin into coenzymes (Fig. 33). The other building units, adenosine triphosphate, phosphoribose pyrophosphate and glutamine are steadily synthesized in normal cells and are present in sufficient amounts to allow the synthesis of adequate amounts of the coenzymes. In human erythrocytes, nicotinic acid is converted into the mononucleotide, which reacts with ATP to form deamino-DPN. Then glutamine serves as an amine donor in the presence of ATP for final synthesis of DPN [94 a]. In the synthesis of coenzyme III, presumably, deamino mononucleotide accepts an extra molecule of phosphate from a phosphate donor to form coenzyme III.

The breakdown of the coenzymes occurs in one of several ways: by nucleoside splitting with separation of free nicotinamide (a in Fig. 31), by deamination of adenine (b in Fig. 31), or by pyrophosphate splitting (c in Fig. 31). The nucleosidase (DPNase, TPNase) is especially important, for it affects only the oxidized form of the coenzymes DPN and TPN, and is inhibited by nicotinamide itself. This property of the enzyme may play a role in the regulation of the concentration of coenzymes within the cells (p. 111) [95].

The chief degradation products of nicotinamide are N-methyl-nicotinamide [96] and N-methyl-6-pyridon-3-carboxylamide [96 a, 97] (Fig. 32). In the presence of a methyl donor such as methionine and of high-energy phosphate, the vitamin can be methylated by active methionine and can then be converted into the 6-pyridon derivative by an aerobic oxidase (quinone oxidase). The methylated product, after treatment with alkali and extraction of butanol, shows intensive blue fluorescence in

ultraviolet light [8, 4]. Nicotinic acid forms only a small portion of the degradation products. Trigonelline used to be considered an important derivative, but is actually ingested in food and is excreted unchanged.

The vitamin is taken in food and is easily absorbed in the intestine as the free acid or as the amide. The form of the vitamin bound as coenzyme is first broken down enzymatically in the intestinal tract and then absorbed. In the blood, 90% of the vitamin is present within the red cells as coenzyme. Chemical determinations give values averaging 438 γ per 100 ml of blood (range 260 to 573 γ) [98]. Microbiological determinations give values of 590 to 930 γ per 100 ml serum, which includes substances which are equivalent to nicotinamide microbiologically [99]. Dunn and Handler determined the amounts of free and bound (90%) vitamin in the tissues [100], and

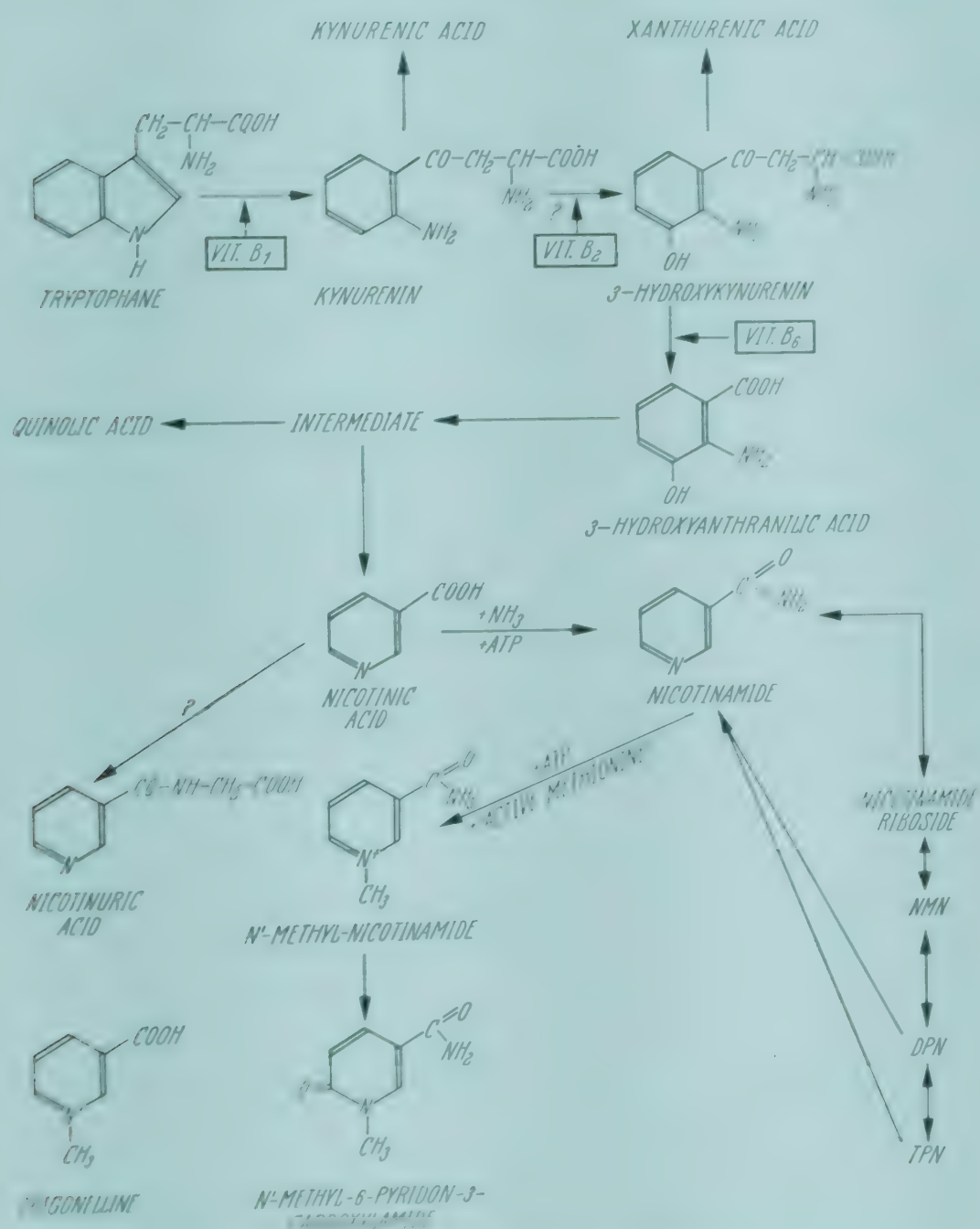


Fig. 32. Synthesis and metabolism of nicotinamide.

found the following average values in the tissues of the dog per gram of fresh weight: liver 153 γ , renal cortex 95 γ , skeletal muscle 71 γ , all expressed as the nicotinamide. Lang [101] gives the following values for the amount of coenzyme (DPN and TPN) per gram fresh weight: muscle 280 γ , liver 245 γ , kidneys 200 γ , heart 190 γ , brain of various species of animals 100 to 130 γ . The vitamin is thus present in the tissues almost entirely as the functionally active form (90%). It is not stored in the body.

The vitamin – that is, the free acid and the amide – is excreted unchanged in the urine only to a small degree. Approximately 10 to 30% appears as the methylated derivative and a like amount as the 6-pyridon derivative or its glycine conjugate [101 a]. Nicotinic acid appears in the urine only after the intake of large amounts of the vitamin. When 12.7 mg of nicotinamide per day were fed to 22 normal persons, 2.5 to 12.2 mg of N-methylnicotinamide appeared daily in the urine [102]. In another study, six persons received an oral dose of 500 mg of nicotinamide and were found to excrete 39 to 113 γ of the methylated derivative per cc of urine [103]. The same authors found that, following the oral administration of 5 grams of D,L-tryptophane, the daily amount of N'-methylnicotinamide in the urine rose from 5.4 mg to 10.3 mg [104]. The normal excretion of the 6-pyridon derivative is 3 to 6 mg per

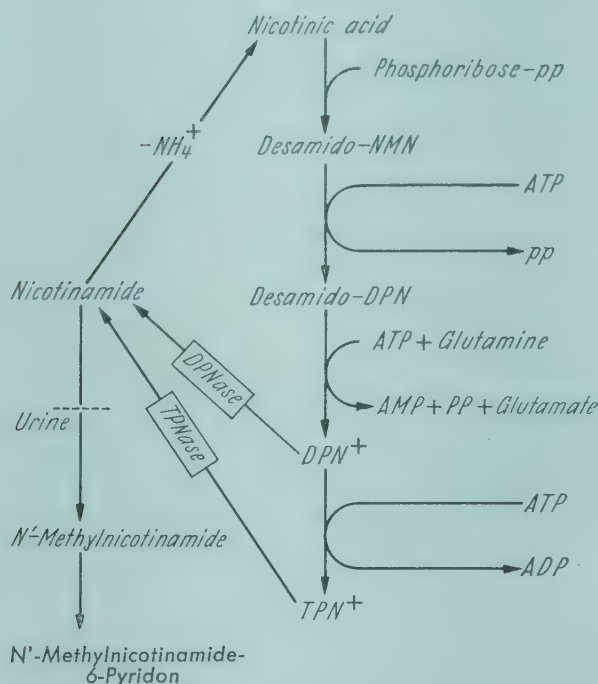


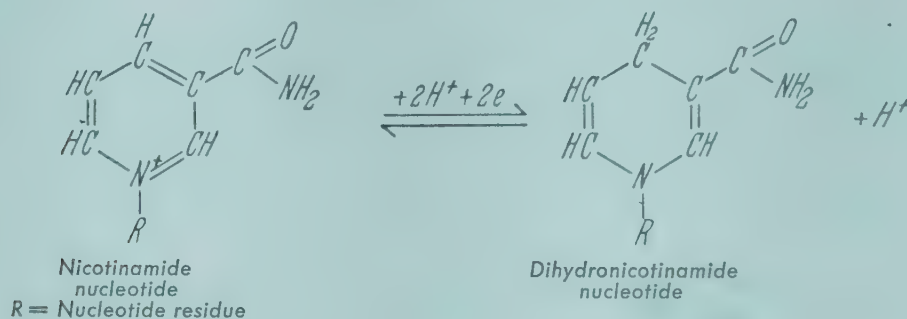
Fig. 33. Synthesis and degradation of pyridine coenzymes (after [94 a]).

pp = pyrophosphate,

NMN = Nicotinic acid mononucleotide.

day [101 a]. This rose to 100 mg per day after a test dose of 600 to 900 mg of nicotinamide.

The function of the vitamin is governed by its pyridine structure. The pentavalent nitrogen of the pyridinium ring in the pyridine coenzymes can be reduced to a trivalent form by the uptake of hydrogen. This reaction is reversible:

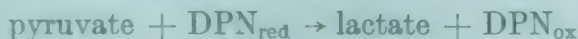


During metabolism, the reduction and oxidation of the ring are catalyzed by enzymes. The active group of the coenzyme is bound to an apo-enzyme (dehydrogenase), at whose surface it reacts with the specific substrate with the exchange of hydrogen.

The substrate is oxidized and the active group of the coenzyme is reduced, or vice versa. A typical example in glycolysis is the oxidation of D-glyceraldehyde-3-phosphate with the aid of a specific glyceraldehyde-3-phosphate dehydrogenase and a pyridine coenzyme to 1,3-diphospho-D-glycerate, after the addition of inorganic phosphate:



The coenzymes are reoxidized enzymatically. The hydrogen may be taken up by another substrate, such as pyruvate, with the aid of another specific dehydrogenase:



Or, the hydrogen may be taken up similarly by the active group of the flavine enzymes



Figure 34 lists various substrates which can exchange their hydrogen with the help of the pyridine group, undergoing oxidation or reduction as the case may be.

This hydrogen transfer by the pyridine enzymes has many applications in metabolic processes. The citric acid cycle proceeds by virtue of dehydrogenations dependent on DPN and TPN. Pyridine enzymes also catalyze various other reactions: synthesis and degradation of amino acids, fatty acids, choline; synthesis of cholesterol from acetyl-CoA; various reactions of the steroids; conversion of orotic acid into dihydro-orotic acid, of retinene into vitamin A, of quinone into hydroquinone, and of sulfhydryl compounds into S-S (disulfide) compounds. As a part of the process of respiration, the pyridine enzymes transfer hydrogen from the substrates to the flavine enzymes and thus initiate the most important energy producing reactions.

The pyridine enzymes are the main hydrogen carriers in the network of the metabolism of carbohydrates, amino acids, fats, lipids and especially the energy-producing reactions of cellular physiology. Within the cell, part of the coenzyme is present in reduced and part in oxidized form. The ratio of the two forms is not constant, but varies with the concentration of the substrate and the rate of

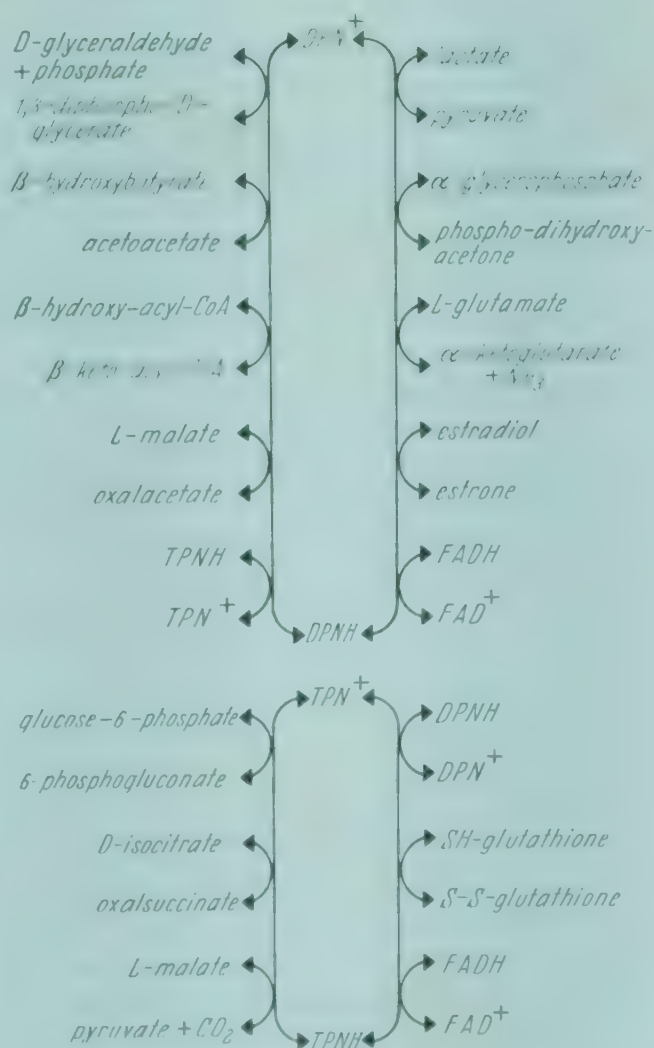


Fig. 34. Some hydrogen transfers which are catalyzed by the pyridine enzymes.

respiration. There is evidence that the ratio DPN^+/DPNH is different from the ratio TPN^+/TPNH [9a, 26a] and also depends on the location in the various cellular compartments [26a]. The ratio apparently determines the direction in which the enzymes transfer hydrogen [105]. The concentration of coenzyme is regulated by nucleosidase [95]. The nucleosidase splits only the oxidized and not the reduced form of the coenzymes; hence the concentration of oxidized coenzyme influences the extent of splitting and therefore the total concentration of the coenzymes. Nicotinamide, the product of splitting, inhibits further splitting.

Pathology of Nicotinamide Deficiency

A deficiency of pyridine nucleotides causes a disturbance of the function of the pyridine enzymes of the body. The resulting clinical picture was described in 1725 by Don Caspar Casal in Spain as "mal de la rosa" (scorbutic leprosy). In 1771, Frappoli first used the common term "pellagra" (pelle agra). Lombroso discovered a relationship between pellagra and corn. Until recently, pellagra was a very widespread disease, although in the last 100 years dietary management has been recognized and has permitted proper treatment.

In 1912, Funk isolated nicotinamide from yeast, without recognizing the relationship of this substance to pellagra [106]. Three years later, however, he proposed the theory that pellagra is a deficiency disease. The work of Goldberger [107] on pellagra-preventing factor (PP-factor) finally placed pellagra among the avitaminoses. In 1937, Elvehjem demonstrated that black tongue of dogs and pellagra of rats responded to treatment with nicotinamide [108]. Fouts [109], Spies [110], Frontali [111] and others showed the excellent response of human pellagra to nicotinamide, and this substance, first synthesized chemically in 1867 by Huber [112], was finally shown to be a vitamin.

Pathogenetically, the clinical picture of pellagra is due to a disturbance of enzymatic functions of the cells. Handler [113] investigated the problems of pathogenesis experimentally, using dogs with typical pellagra due to deficiency of nicotinamide in the diet. A few weeks after the experimental diet is started, black tongue appears together with changes in the buccal mucosa, the gums, and the base of the tongue. The oral mucosa becomes red, swollen, and ulcerated. Black pigment appears in the center of the tongue. The mucosa of the esophagus and the entire intestinal tract takes part in these changes. Disturbances of the nerves appear. Blood studies show acidosis, increase of the total serum protein, leukopenia and anemia. The dog dies in this first phase because of dehydration. Administration of nicotinamide promptly cures the disease.

Handler investigated the influence of salt solutions on the severe dehydration which occurs in pellagra and found that infusions of physiological saline alone could reverse the abnormalities even without the administration of nicotinamide. If, however, the deficiency diet is continued together with the infusions, the dog develops weakness, loss of weight, and paralysis, and ultimately dies after several months. This second phase of the illness can also be cured by administering the vitamin. The saline infusion perhaps relieves the initial signs of deficiency by supplying a temporary substitute for a special metabolic function of the vitamin; ultimately, however, other signs of the deficiency supervene and lead to death. During the first phase, the coenzyme content of the muscles, the red cells, and the other body tissues is not

particularly changed. The liver is an exception, but only to a small degree. In the second phase, there is a marked fall of the content of pyridine coenzymes in the red cells, in the muscles (up to 60%), and in other tissues, and the biochemical manifestations of deficiency become prominent. In accordance with the different sensitivities of the pyridine enzymes of particular tissues, each tissue in turn sooner or later shows biochemical damage due to the deficiency and ultimately shows irreversible anatomic changes. The clinical picture is often the same as that seen in other vitamin deficiencies. In general, disturbance of the pyridine enzymes becomes most evident at those tissues which are especially sensitive to lack of oxygen, notably the central nervous system and the adrenal glands.

The signs of the first phase of the illness shown by Handler's dogs are similar to those seen in disturbances of adrenal function. This fact suggested that the cause of death at this stage might be disturbed adrenal function. Handler noted the similarity between the symptoms of pellagra and those of Addison's disease: both disorders show low blood pressure, similar changes of the electrocardiogram, similar alterations of salt and water content, and pigmentation. Cases are seen in clinical practice which are first diagnosed Addison's disease and treated as such, but fail to respond until treatment with nicotinamide is begun. Furthermore, cases of pellagra have been described which showed changes in the adrenals [114].

The cause of the dehydration, blood changes, and mucosal changes observed by Handler is deficient function of those pyridine enzymes which are especially sensitive to deficiency of nicotinamide. In comparison to other dehydrogenases of the body, these enzymes require unusually high amounts of nicotinamide coenzyme. Nothing is known concerning the particular dehydrogenase involved, that is concerning the substrates with which the enzymes especially sensitive to coenzyme deficiency react. The adrenal glands, which control the salt and water content of the body, are of importance in this regard. The synthesis of the adrenal cortical hormones from cetyl-CoA (Figure 35) is catalyzed at two sites by pyridine enzymes. In addition, as is well known, the adrenal glands have a great requirement for oxygen and, like the brain, are extremely sensitive to oxygen lack.

Confirmation of the above pathogenetic explanation of experimental pellagra in dogs has not yet been made. The relationship between the concentration of pyridine nucleotides in the adrenal cortex and normal or impaired adrenal function is not known. However, the above discussion affords an example of the biochemical investigation of a disease from primary cause to clinical picture.

Human pellagra is similar to the pellagra of dogs in many respects. The concentration of the coenzymes in the muscles and the blood is markedly reduced in accordance with the phase of the disease. There are no special studies concerning the salt and

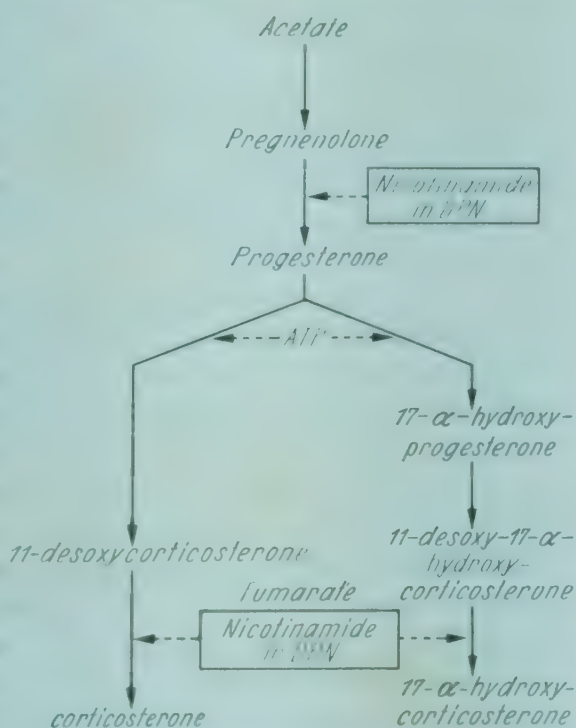


Fig. 35. The dependence of adrenal steroid synthesis on nicotinamide (DPN). From Staudinger [115].

water content. It is questionable if the mucous membrane changes can be explained on the basis of adrenal malfunction even though the resemblance of the picture to that of Addison's disease suggests this possibility.

Various authors have suggested that the central nervous system lesions are due to accompanying beri-beri. However, these abnormalities are not corrected by thiamin, but by niacin. Jolliffe and his coworkers [116] believe that encephalopathy of pellagra can develop so rapidly that death may occur before the skin and mucous membrane lesions have time to occur. In considering the relationship between the pyridine enzymes and the metabolism of the brain, it must be remembered that the brain contains a highly concentrated nucleosidase, which indicates a high turnover of coenzymes; and that the only substrate used by the brain is glucose, whose oxidation requires an especially high amount of pyridine coenzymes. Fundamental is the finding that the brain is very sensitive to lack of oxygen, and is thus strictly dependent on a completely normal cellular respiration. Strughold [117] stresses these interrelationships. It is not clear why pellagra damages the brain and beri-beri primarily the peripheral nervous system. The reduced degradation of pyruvate and α -keto glutarate in the brain in human beri-beri is apparently not as pathogenic as lack of nicotinamide.

Cardiac muscle is a sensitive indicator of normal nicotinic acid intake, requiring large amounts of coenzyme for normal function. When the heart muscle of a rabbit has been damaged, the administration of small amounts of nicotinic acid improves cardiac action and the electrocardiogram [118]. The electrocardiographic changes in human pellagra (p. 117) are an expression of the deficient supply of energy to the cardiac muscle. The EKG changes are similar to those seen in Addison's disease and again support the hypothesis of adrenal dysfunction in pellagra.

In the liver, deficiency of nicotinamide first manifests itself at an advanced stage of the disease: anatomically there is fatty degeneration, and biochemically there is a reduction of the content of coenzyme. There is an increased excretion of coproporphyrin III in the urine. Spies [119] considers the excretion of this pigment as a diagnostic *sine qua non* for this deficiency. However, it does not occur until the damage to the liver has become obvious. Coproporphyrin III excretion is seen regularly in cases of alcoholism associated with pellagra. The pigment is seen in many liver disorders, both primary liver diseases and diseases which are secondary to disturbances of the intestinal tract, infections, malnutrition, etc. The excretion of the pigment is thus not pathognomonic for pellagra.

The cause of the skin changes of pellagra is not known. The changes may be accelerated by sunlight, X-rays, and heat. Similarly, sunlight encourages the appearance of the other manifestations, such as vomiting, loss of appetite, diarrhea, and psychic disturbances. No special relationship has been found between sunlight and porphyrin metabolism, which was formerly considered the cause of pellagrous dermatitis in analogy with the congenital porphyrias and because of the favorable effect of nicotinic acid on dermatoses due to sunlight.

Experimental pellagra in rats and roosters is of interest from the point of view of tryptophane. If such pellagrous animals are fed tryptophane, they excrete nicotinic acid [6] and the signs of pellagra are improved. As is well known, bacteria can readily convert tryptophane into nicotinic acid (Fig. 32), and the improvement of the symptoms of pellagra and excretion of nicotinic acid are explained by conversion of ingested tryptophane to nicotinic acid by intestinal bacteria. Man must ingest the vitamin itself, although he can derive some from ingested tryptophane. Deficiency of the vitamin can therefore be aggravated by a deficiency of tryptophane or protein

in the diet. Corn contains very little nicotinamide, and, in addition, the protein of corn is poor in tryptophane [120], hence the relationship between corn diet and pellagra. Tryptophane has a second function as an essential amino acid and as a building stone of enzyme protein (e. g., lactate dehydrogenase) (p. 108). The actual biochemical events are complicated by the fact that the conversion of tryptophane into nicotinamide requires vitamins B₆ and B₂ (Fig. 32). The functions of most of the B vitamins in metabolism are closely interrelated (Fig. 41), and individual B-avitaminoses are modified by the actions of other vitamins. Thus, for example, the administration of large amounts of vitamin B₁ may result in secondary pellagra because of an increased requirement for pyridine enzymes due to the increased turnover of vitamin B₁ in metabolism [121].

A word should be said concerning the therapeutic use of *antimetabolites* or anti-vitamins. Woolley [122] used a structural analog of nicotinamide, 3-acetylpyridine, to produce in mice a pellagra which resembles the pellagra found in dogs and man; niacin cured the resulting disease. Because of its chemical relationship to nicotinamide this type of antivitamin is incorporated into the pyridine coenzymes instead of the vitamin itself. However, this "false coenzyme" cannot undergo oxidation and reduction, so that the manifestations of coenzyme deficiency follow. Certain antivitamins are widely used in the therapy of infectious diseases. Too high doses of isonicotinic acid hydrazide and semicarbazone derivatives, for example, can dislodge nicotinamide from the coenzymes. This mechanism of action of the antibiotics makes them effective antibacterial agents, but excessive and prolonged administration may have a bad effect on human metabolism with the development of pellagra-like symptoms and signs.

Human Pellagra — Clinical Considerations

Pellagra is especially prevalent among the poor of all ages and both sexes. Its occurrence is usually associated with the use of corn diets (maize), especially when corn, which is rich in carbohydrate but poor in protein and vitamins, is the chief or only source of nourishment. The disease occurs endemically in tropical and subtropical climates and in the middle East, Rumania, Spain, Italy, and southern France. In 1906, over 100,000 cases of pellagra were seen in Rumania. Today the disorder is seen in large cities and industrial areas, usually sporadically especially in spring and early summer.

Endemic pellagra is the true disease due to deficiency of the vitamin in the diet, and is seen chiefly among the poor and in times of war and starvation. *Pellagra* may also occur as a *secondary* phenomenon in many other diseases. Thus, there is an increased requirement for nicotinamide in pregnancy, diabetes, hyperthyroidism, infectious diseases, febrile illnesses, leukemia, Addison's disease, most disorders with elevated metabolic rates, nutritional fads (unequal composition of food protein with regard to amino acids increases the requirement for nicotinamide), physical exertion, and exposure to x rays. The uptake of the vitamin may be impaired when there is faulty absorption and overgrowth of intestinal bacteria, as in chronic diarrheas, sprue, and celiac disease. In addition, secondary pellagra may be seen when the vitamin is insufficiently utilized in cellular metabolism as a result of parenchymal damage, especially in cirrhosis of the liver (reduced synthesis of coenzymes from the vitamins and disturbed synthesis of enzyme proteins).

Since pellagra usually is not an isolated deficiency of nicotinamide but is associated with other deficiencies or generalized malnutrition, the clinical symptomatology is

not always classical. Age, body constitution, and previous state of nutrition and health influence the symptoms and signs of the disease. Pellagra is different in a patient with diabetes from that in malnutrition and in normal carbohydrate nutrition. The site of the chief symptoms and signs differs in these different situations: skin, intestinal tract, central nervous system. The more chronic form presents anew from year to year, usually in spring. In the acute form, death may occur rapidly.

The *prodromata*, which usually last for weeks, and the latent form of the disorder begin with nervous and psychic symptoms, with the gradual development of lassitude, sleepiness, pains in the extremities, insomnia, easy fatigability, lack of concentration, forgetfulness, depression, headache, and backache. Later there are loss of appetite, burning of the tongue, pyrosis, diarrhea, abdominal pain, vomiting, and loss of weight. Some patients complain chiefly of muscular pain with inability to walk, pain in the legs, cold feet, and paresthesias [114].

In the classical case of pellagra, the prodromal symptoms are followed by manifestations of involvement of the nervous system, the mucous surfaces, and the skin. The psychiatric and neurologic signs of involvement of the nervous system are multiple, and together are called pellagrous encephalopathy [116]. There is a breakdown of the normal personality, with the occurrence of apathy, lethargy, depression, stupor, and progressive mental deterioration with or without hallucinations. Coma and fever may occur prior to death.

At the same time, signs of extrapyramidal and peripheral nervous system involvement appear: hypertonicity, choreiform movements, appearance of sucking and grip reflexes, and reduced or exaggerated pyramidal signs. In addition, there may be spastic hemi- or diplegia, signs of peripheral neuritis, signs which simulate posterolateral sclerosis, and retrobulbar neuritis. Pellagrous encephalopathy occurs especially in alcoholic patients and in malnourished individuals, as in times of war. Pellagrous changes of the skin and the intestinal tract are usually only suggestive of the diagnosis. Delirium tremens may be the presenting symptom in alcoholics, although at times they complain of acute stomatitis and gastrointestinal symptoms.

In most instances, mucous membrane changes are already present during the prodromal period of the disease. The tongue and the mouth burn, and appetite is lost. The tongue becomes reddened, first at the tip and the edges, and later all over. The tongue becomes swollen. The pronounced glossitis with swollen papillae is known as "raspberry tongue." The stomatitis and gingivitis cause severe pain and increased, tenacious salivation. Desquamation, pigmentation, and ulceration of the tongue may occur and result in atrophy of the mucous membrane (Fig. 36). The pharynx and the esophagus show inflammation. Heartburn is pronounced, especially in children, and vomiting occurs. Watery diarrhea occurs early in the disease but there may be alternating diarrhea and constipation. Anacidity and histamine-free achlorhydria may be present, and the secretion of pancreatic enzymes is reduced. The mucous membrane lesions lead to marked loss of weight and increasing debility. In females, vaginitis forms a part of the clinical picture.

The skin changes of pellagra (Fig. 37) begin with transient symmetrical erythematous lesions at the exposed parts of the body, at sites exposed to trauma, at the breasts, the genital folds, and the subscrotal areas. There is a sharp line of demarcation between the redness of the pellagrous lesion and the surrounding skin. Swelling, vesicles, and ulceration occur. In its full-blown phase, the areas of erythema are dark-red in appearance, so that the patient looks as if he has a severe sunburn. The dark-red areas subsequently become confluent and turn brown. The skin burns,

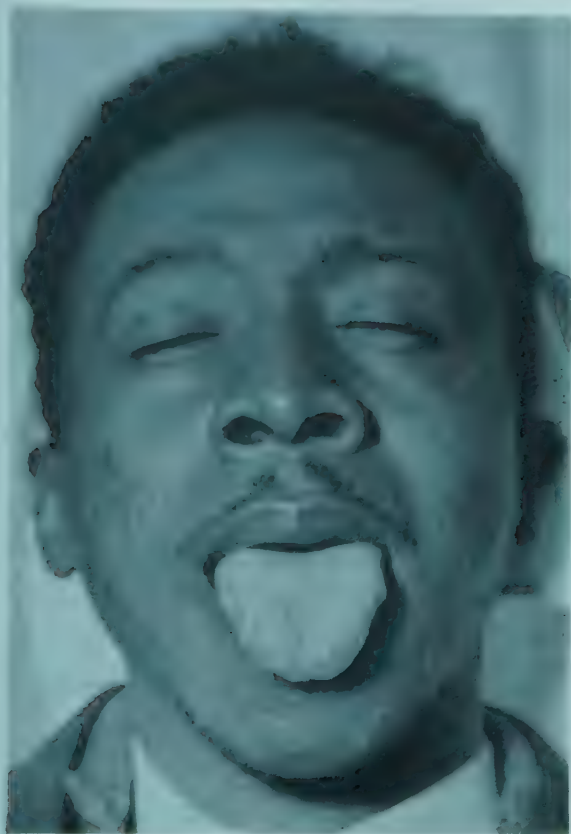


Fig. 36. Changes of the tongue in pellagra.
(Case of Dr. Tom Spies.)



Fig. 37. Changes of the skin in pellagra.
(Case of Dr. Tom Spies.)

itches, begins to desquamate, becomes thickened, and shows rhagades. Infection may supervene, or atrophy may occur. Hyperkeratosis may occur in certain areas such as the nasolabial folds and the tip of the nose and elbows. The nails may show trophic changes.

There is less involvement of the heart and the circulation. The blood pressure is low. The electrocardiogram shows flattening or inversion of the T-wave, usually a prolongation of the QT-interval, and sometimes low voltage. If the heart is previously diseased, pellagra further damages the heart. Kidney lesions do not occur in pellagra, but marked diuresis may be present. The bone marrow almost always reacts after a few weeks, especially in alcoholics, with the development of either hypochromic or macrocytic anemia, sometimes with megaloblastic erythropoiesis.

The diagnosis of pellagra is quite simple in those cases in which the typical signs of involvement of the skin and the intestinal tract are present. Psychic and neurological involvement complicate the picture. It is important to take a good dietary history. In cases of doubt, a therapeutic trial with nicotinamide in large doses makes the diagnosis certain, but such therapy may fail in far advanced cases. Differential diagnosis of pellagrous lesions of the central nervous system must be made from disorders of the spinal cord, especially when psychic symptoms are lacking. This differentiation can often be made only by the dietary history. The peripheral neurological signs may simulate posterolateral sclerosis; study of the blood and appropriate therapy will permit proper diagnosis. Wernicke's syndrome and Korsakoff's psychosis in alcoholic patients are more readily related to beri beri than pellagra. Differentiation of pellagra from beri-beri may be accomplished by determination of the level of pyruvate in the blood (p. 100), which rises in beri beri. Coproporphyrin III is increased in the urine in

patients with liver involvement (especially alcoholics), but this substance is not pathognomonic (p. 114). The cerebrospinal fluid is normal. The nicotinamide content of the normal blood, which measures 0.25–0.89 mg%, shows such marked variations that it cannot be used as a criterion of deficiency of the vitamin in the body.

Deficiency of nicotinamide in the body can be demonstrated by a saturation test. When the body is saturated with the vitamin, it excretes any excess vitamin which is administered. Retention of a dose indicates lack of saturation – i. e., deficiency. Handler [113] has described a loading test using nicotinamide which gives an approximation of the degree of saturation of the body with the vitamin and which can be used for diagnosis. In the test, a dose of 100 to 500 mg of nicotinamide is given and the excretion of N-methylnicotinamide is measured in the urine. The total amount of the administered dose is excreted in the urine in methylated form within 2 to 24 hours unless there is a latent or obvious deficiency of nicotinamide [4, 8].

Pellagra is treated by the administration of large amounts of nicotinamide, preferably together with all the members of the vitamin B-complex and a diet rich in protein and carbohydrates (Tabel 39) [371]. The usual daily dose is 50 to 500 mg by mouth; in severe cases, the dose can be 1,000 mg intravenously and 500 mg intramuscularly daily for the first 10 days. Parenteral administration is recommended when intestinal absorption may be faulty because of involvement of the intestinal mucosa. Only nicotinamide can be given intravenously, for the free acid itself causes undesirable side reactions. Larger doses are necessary for pellagrous encephalopathy (1,500 mg daily). The vitamin is continued in therapeutic amounts in each case until clinical improvement has occurred and the body has been saturated with the vitamin, and then normal doses are resumed. Of course, nicotinamide is not used alone but only in combination with all the other B-vitamins and with a good diet. Treatment of underlying diseases is especially important. The prognosis depends on the stage of the disease when treatment is started. Since the use of nicotinamide, the prognosis is generally excellent. In cases with cerebral lesions, cure is possible only to the degree that anatomic alterations have not yet occurred.

The Flavine Enzymes

Physiology of Riboflavin and its Enzymes

In 1925, Bleyer and Kallmann discovered a yellow substance, “lactochrome,” in certain grains (whey) [123]. In 1932, Szent-Györgyi and Banga [124] found that cardiac muscle contains a yellow pigment, “cytoflave,” which has the property that it is colorless when reduced and yellow when oxidized. In the same year, Warburg and Christian [33] discovered “old yellow enzyme” in yeast, and subsequently a number of other yellow enzymes were discovered and described [30]. It was soon found that the yellow component of all these substances was the same. Warburg had already found [33] that the active group of the yellow enzyme was a nitrogen-containing ring compound, alloxazine. Kuhn et al [125] synthesized alloxazine and its derivatives and determined its structure. Finally, Theorell [126] was able to determine the type of binding of the active group within the yellow enzyme. He broke the enzyme down into two components – a protein and the yellow prosthetic group – and showed that alloxazine was bound as a nucleotide (flavinmononucleotide). He was also able to

Table 36. Crystalline Yellow Enzymes with Flavin-adenine dinucleotide as the Coenzyme (B a. 5)

Enzyme	Substrate
DPN-cytochrome reductase	DPNH
TPN-cytochrome reductase	TPNH
Xanthine oxidase	xanthine hypoxanthine aldehyde xanthopterin
Glycine oxidase	glycine
L-amino acid oxidase	L-amino acids
D-amino acid oxidase	D-amino acids
Acyl-coenzyme A dehydrogenase	acyl-coenzyme A
Vitamin K ₁ reductase	vitamin K ₁

recombine the 2 components to re-form the original enzyme. Kuhn and Rudy [127] then synthesized and determined the structure of the nucleotide.

Like the proteins of the pyridine enzymes, the protein components of the yellow enzymes – the apoenzymes – are pure proteins. Some of the yellow enzymes have been prepared in crystalline form (Table 36). However, in no case is the arrangement of the amino acids within the enzymes known. Studies of the effect of deficiencies of essential amino acids on the enzyme content of rat liver have shown that a deficiency of tryptophane and methionine leads to marked reduction of the activity of xanthine oxidase, a yellow enzyme which oxidizes xanthine to uric acid [62]. A protein-poor diet causes a reduction in the tissue content of the yellow enzymes [101].

The prosthetic group of the yellow enzymes may be a mononucleotide (flavin-mononucleotide, FMN, riboflavin-5'-phosphoric acid) or a dinucleotide (flavin-adenine dinucleotide, FAD) (Fig. 38). The active group is the substituted isoalloxazine nucleus, whose yellow color gives its name to these enzymes. The compound formed from this pigment with ribitol is riboflavin, also known as lactoflavin and vitamin B₂. Chemically, it is 6,7-dimethyl-6-D-ribityl-isoalloxazine. Riboflavin is relatively insoluble in water and alcohol. It is stable against heat and oxidation and shows

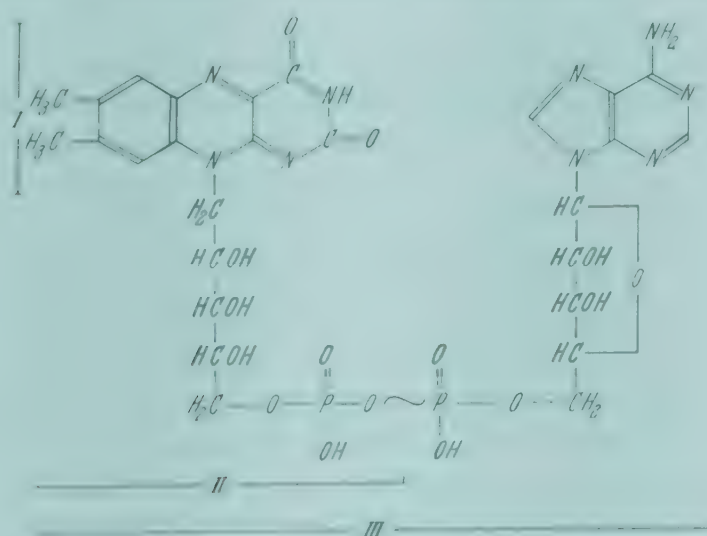
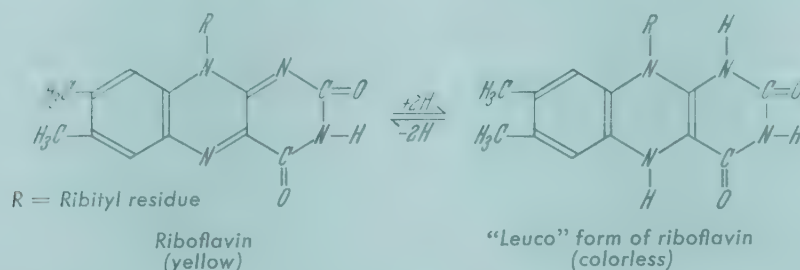


Fig. 38. Structural formula of the flavine coenzymes.

I. I. Isoalloxazine. II. FMN (flavin mononucleotide). III. FAD (flavin-adenine dinucleotide).
~ P High-energy phosphate bond.

fluorescence in blue and ultraviolet light. It is extremely sensitive to light. When exposed to light in neutral solution, the sugar is split off and lumichrome (6,7 dimethyl alloxazine) is formed. In alkaline solution, the chief product is lumiflavin (6,7, 9 trimethyl isoalloxazine). In these processes, the vitamin loses its biological activity. The fact that the vitamin fluoresces is the basis for one method of determination [4, 8, 370]. Microbiological assay can also be performed.

Riboflavin can be reduced reversibly to a colorless compound, and this property is the basis of its function in cell metabolism as a redox catalyst. The reaction produces dihydroriboflavin by the incorporation of hydrogen in positions 1 and 10:



Riboflavin is found in nature in milk, liver, kidneys, cardiac muscle, yeast, tumor cells, germinal seeds, et al. It is present free and also in a bound form as riboflavin-5'-phosphate (FMN) and flavin-adenine-dinucleotide (FAD). Both bound forms function as coenzymes in metabolism and are largely bound to a protein carrier. Each of the forms of the yellow coenzymes can be used by the human body as a vitamin, for the process of digestion sets riboflavin free from its bound forms.

The biological synthesis of riboflavin is as yet unknown. It can be synthesized by intestinal bacteria (*B. proteus*, *B. aerogenes*, *B. mesentericus*, *B. vulgatus*, *B. fecalis alcaligenes*, *E. coli*) and by many other microorganisms and plants [128]. More highly developed forms of life cannot synthesize the vitamin. The human requirement is covered by riboflavin taken in in the diet and, to a small degree, by intestinal bacterial synthesis.

The pathways of synthesis and catabolism of the coenzymes in the body are shown in Fig. 39. The synthesis of the dinucleotide from riboflavin can be readily followed in, for example, human erythrocytes both in vitro and in vivo. Catabolism of the nucleotide is accomplished by nucleotido-pyrophosphatase and phosphatases. The vitamin is broken down in the colon, but the pathway of degradation is not yet known.

In the blood, the vitamin is found bound chiefly within the blood cells. Human serum contains the vitamin as free riboflavin and FMN (total 0.1 to 1.3 $\gamma\%$, with an average of 0.8 $\gamma\%$), and as FAD (1.8 to 3.0 $\gamma\%$, with an average of 2.4 $\gamma\%$). The total serum riboflavin is approximately 3.2 $\gamma\%$ [129]. Microbiological assay has given a value of 50 $\gamma\%$ for the total riboflavin [130]. The content of leucocytes is 252 (232 to 293) $\gamma\%$, and that of the red cells is 22.4 (18.0–26.2 $\gamma\%$) [129]. In the tissues, 70 to 90% of the vitamin is present as the dinucleotide (FAD). Raffay [131] determined the concentration of riboflavin in 3 adults, and gives the following results in γ per gram of fresh weight: heart, 5–6.8; lungs, 1.3–1.6; kidneys, 5.3–11; brain, 1.4–4.6;

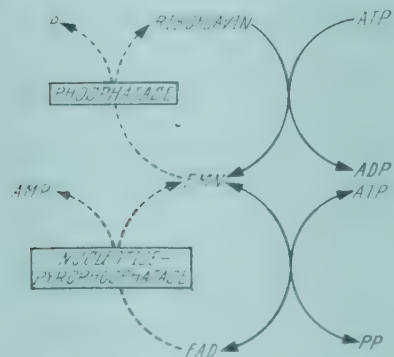


Fig. 39. Synthesis and degradation of the flavin coenzymes. Hypothetical reactions are shown in dotted lines.

liver, 6.9-8.0. Tadhé et al. [132] found 16 to 18 γ riboflavin per gram of fresh weight of liver.

After separation of riboflavin from its bound form, the vitamin is excreted chiefly in the urine and to a small degree in the bile. The amount excreted in the stool (30 to 50%) includes the vitamin synthesized by the intestinal bacteria. Balance studies [133] show that the daily urinary excretion depends on the oral dose: when the daily oral dose is 0.161 mg, the excretion is 2.8%; when 1.017 mg, 11.8%; when 1.234 mg, 14.3%; when 7.164, 40% [166] (Table 37). The smaller the dose ingested, the greater

Table 37. The excretion of riboflavin in the urine as a function of the oral intake of riboflavin [166]

Oral intake of riboflavin, mg/day	Urinary excretion of riboflavin, γ /day
0.79	70
1.04	160
1.26	130
1.62	320
2.23	1180
2.72	1310

the percentage retained within the body. In the rat on a deficient intake of riboflavin, the excretion can fall to 2% of the normal excretion. When the vitamin is then administered again, there is a rapid increase in the excretion - an indication that there is little or no storage of the material. Saturation of the body with riboflavin is rapidly reached. The general state of nutrition and the composition of the diet influence the extent of retention of the vitamin. Simultaneous administration of casein increases the uptake of riboflavin in the liver. Thirty percent of this action is due to the methionine content of casein, for methionine itself increases the uptake of riboflavin in the liver [134]. In protein deficiency, the bound riboflavin of the liver is broken down and excreted in the urine, while the concentration of free riboflavin in the liver remains relatively constant. The liver and the muscle can no longer synthesize the dinucleotide (FAD). In protein deficiency, therefore, the excretion of total riboflavin in the urine goes up [135].

The degree of saturation of the body can be estimated by a loading test. Various methods have been described. Robinson [6] believes that, when the saturation of the body is normal, the 24-hour urine shows not less than 200 γ on a normal diet, and not less than 20% of a test dose of 1 to 10 mg.

In the growing mammal and in microorganisms riboflavin is a growth factor. Riboflavin is the simplest of all the naturally-occurring growth factors. A number of other compounds which are structural analogs of riboflavin and differ only slightly from it have the same qualitative action, but never show it to the same degree.

With regard to its biological role, the structure of the vitamin is not particularly specific. Thus, the ribitol can be replaced by arabinose without any loss of biological activity, and the dimethyl benzol ring can be detached without loss of biological activity; but both events cannot occur at the same time. Substitution at position 3 causes loss of all activity of the vitamin. Other structural analogs act as antivitamins (antimetabolites) - for example, 6,7 dichloro-6-D-riboflavin - and inhibit the action of riboflavin in growth or enzymic experiments [136]. The antimalarial drugs atabrine and quinine belong to the antivitamin group.

In the fully active yellow enzymes, the flavin nucleotides are prosthetic groups which are firmly bound to a protein component. The dissociation constant is an expression of the degree of firmness of this binding between prosthetic group and protein. For cytochrome-c-reductase, which is a yellow enzyme of respiration, the dissociation constant is 10^{-9} mols liter. This signifies that only a very small portion of the prosthetic group is present in free form. While the pyridine nucleotides can be exchanged among the various apo-enzymes, the flavin nucleotides are fixed to one apo-enzyme. For this reason, the latter are called prosthetic groups rather than, as is the case with the pyridine nucleotides, coenzymes.

The prosthetic group is bound to the protein by the imido group (position 3) of alloxazine and the phosphoric acid residue. The imido group is essential for the occurrence of this binding and therefore also for the physiologic activity of the vitamin. If it is altered (for example, by methylation to 3-methyl-riboflavin), the biological activity, as determined by the growth test, is lost. The binding of the prosthetic group to the apo-enzyme causes a change of the redox potential of the vitamin. This change is important for the enzymic activity of the vitamin. Thus, if the free nucleotide and the vitamin have a redox potential of $E'_0 = -0.185$ V at pH 7.0, the protein-bound nucleotide of Warburg and Christian's "old yellow enzyme" has a redox potential of $E'_0 = -0.06$ V at pH 7.0 [137]. The potential is thus shifted to a thermodynamically favorable position between the pyridine enzymes and cytochrome, with which the yellow enzymes can react. Hydrogen donors for the flavine enzymes DPN-cytochrome-c-reductase and TPN-cytochrome-c-reductase are the pyridine enzymes; hydrogen acceptors are the cytochromes. Disturbances of the yellow enzymes thus cause disturbances of the pyridine enzymes or the iron-containing cytochromes, and vice versa.

The flavine enzymes react not only with hydrogen donors such as the pyridine enzymes, but also directly with the substrates of metabolism. Flavine enzymes include, among others, D- and L-amino-acid-dehydrogenases, diamino-oxidase (histaminase), xanthine oxidase, aldehyde oxidase, quinone oxidase, acyl-CoA-dehydrogenase, and succinate oxidase (fumarate reductase) (Table 36).

Pathology of Riboflavin Deficiency

In 1916, vitamin B was considered to be a chemically homogeneous substance [138]. However, as time passed, it became obvious that several different substances were actually present in "vitamin B." Vitamin B₂ (riboflavin) was discovered following the work of Goldberger and Lilly on pellagra [139], and the nature and function of nicotinamide and vitamin B₆ were determined. Goldberger and Lilly, working on rats, produced a deficiency syndrome characterized by eye signs and loss of hair; this syndrome could be prevented by the use of a heat-stable factor of the vitamin B complex. Subsequent workers first disagreed on the nature of the heat-stable factor, but finally established two important functions, a growth function and an anti-dermatitic function. The anti-dermatitic factor is related to vitamin B₁, and the growth factor is identical with riboflavin. In 1935, it was finally shown that riboflavin is not related to pellagra [108].

It was not possible to produce experimental deficiency of riboflavin in animals until after the chemical structure and the synthesis of riboflavin had been determined. Riboflavin-free diets were impossible before this time. Feeding such diets to laboratory

animals resulted in disturbances in practically all tissues of the body. The rat showed loss of hair, dermatitis, conjunctivitis, corneal damage, peripheral parylyses, muscular degeneration, leukopenia with relative or absolute lymphocytosis (an early sign, prior to the eye lesions), and hypoplasia of the marrow with anemia. Anatomic changes occurred in the thyroid, the adrenal cortex, the testes (atrophy), and the thymus (early involution) [140]. The fetus of a mother with riboflavin deficiency showed disturbed development, including impaired growth of the extremities, syndactyly, and cleft palate. More prolonged deficiency resulted in abortion or sterility. In dogs, the chief findings include intestinal disturbances (vomiting and diarrhea) and fatty degeneration of the liver. In contrast to vitamin B₁-deficiency, riboflavin deficiency shows no anorexia. In young animals, especially rats, the chief finding is cessation of growth. Diminished excretion of riboflavin precedes the appearance of the clinical signs.

Deficiency of riboflavin leads to a fall of the concentration of riboflavin in all the tissues. The concentration of flavin-adenine-dinucleotide is especially reduced in the heart and the liver [141]. The vitamin content of the rat cornea, which is normally high, also falls in riboflavin deficiency. There is a reduction of xanthine oxidase in the liver (xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid), and feeding of riboflavin promptly eliminates the abnormality [142]. The activity of the D-amino acid oxidases of the liver and kidneys is also reduced. There are, in addition, disturbances of glucose metabolism in riboflavin deficiency [143, 144]. With regard to fat metabolism, there are no experimental data regarding the effect of riboflavin deficiency, but it must be assumed that the β -oxidation of the fatty acids, in which the yellow enzymes act as hydrogen acceptors, is disturbed.

Changes in the hormone metabolism of the adrenal glands are reflected in anatomic changes of the adrenal cortex in ariboflavinotic rats [140]. Other signs of adrenal cortical involvement are the low blood pressure, the tendency to shock, and the intestinal disturbances in the experimental dog. The inactivation of estrogens by the rat liver requires the action of the yellow enzymes, but it is not yet known how riboflavin deficiency leads to impairment of this inactivating function. The effect of riboflavin deficiency on the body proteins might be the result of the relationship of the vitamin to the amino acid oxidases. The excretion of ingested amino acids is increased. Impaired catabolism of amino acids may have toxic effects, which can be induced by the feeding of various amino acids to animals with riboflavin-poor diets (L-cystine, D, L-tryptophane, L-tyrosine, L-histidine, glycine, D-glutamic acid) [145].

In the isolated tissue, deficiency of riboflavin is manifested by a reduced utilization of oxygen [146]. The basal metabolism, however, is not altered. Hydrochloric acid production and vision are impaired. The normal gastric mucosa contains large amounts of riboflavin and its nucleotides (10.9 γ riboflavin per gram fresh weight and 9.2 γ riboflavin nucleotide per gram fresh weight [147]). Anacidity is an early sign. Apparently, the yellow enzyme is essential for the production of hydrochloric acid. The anacidity may also be one of the causes of the anemia which later develops. With regard to vision, certain observations suggest that riboflavin functions as an acceptor of light in the eye. While vitamin A is concerned with the rhodopsin mechanism of dark adaptation [148], riboflavin seems to permit adaptation to light on the part of the retinal pigment. This pigment has the physical property of converting short-wave light into green-yellow fluorescence, for which the eye has a special sensitivity [149]. In addition, riboflavin protects retina and cornea against light (the total flavin content of the cornea is 181 γ°) [150]. Riboflavin is also present in the lacrimal glands and in the tears. Riboflavin deficiency is accompanied by corneal disturbances.

marked photosensitivity, and poor dark adaptation. The disturbances of production of the tears are especially prominent in Sjörgens's syndrome, in which Vannotti showed deficiency of riboflavin [9].

When the diet is deficient in protein, the activity of the yellow enzymes diminishes in all tissues. Deficiency of tryptophane causes marked diminution of xanthine oxidase activity and succinate oxidase activity, with the result that the oxygen uptake of the rat liver is diminished. When the diet lacks methionine, there is almost complete disappearance of xanthine oxidase [62]. Intestinal disturbances are associated with impaired absorption of vitamin. In diabetes mellitus, the cells are said to lose the ability to bind lactoflavin (riboflavin) [144]. In hyperthyroidism, the vitamin may fail to undergo normal phosphorylation [151]. In contrast to a generalized lack or deficiency of flavine enzymes is a localized deficiency of one flavine enzyme, hemoglobin reductase, in the erythrocytes as seen in an inborn error of metabolism known as familial hemoglobinemia (methemoglobinemia) [151 a].

Clinical Ariboflavinosis

There are few data concerning the occurrence and incidence of this relatively new disorder. Sebrell [152], Spies [153], and Vannotti [9] have described the clinical picture in detail. The disease occurs chiefly together with other deficiency diseases, usually among poor people, in large industrial cities, and in association with unbalanced diets. Other symptoms and signs may mask those of ariboflavinosis itself. Vannotti [9] also noted the frequent occurrence of impaired absorption of riboflavin in chronic intestinal disorders with atrophy of the mucosa, and in sprue and idiopathic steatorrhea. Liver disease prevents the intracellular transfer of the vitamin into the functional group of the yellow enzymes. Certain diseases increase the amount of oxidation in the body and therefore the requirement for vitamins: fever, infections, tumors, tuberculosis, hyperthyroidism, chronic alcoholism, pregnancy, lactation, and growth.

Sebrell and Butler [152] studied human ariboflavinosis in volunteers on deficiency diets. The prodromal period is identical with that of beri-beri or pellagra (p. 132, 155). The mouth soon becomes dry and sensitive. The tongue begins to burn and the corners of the mouth itch. The patient complains of poor digestion, bloating, and ultimately poor vision (photosensitivity) and burning of the eyes. The complaints come and go, at first without objective findings. Ultimately, however, typical changes occur, notably *cheilosis*, *atrophic glossitis*, *seborrheic dermatitis* (especially at the lips), and *keratoconjunctivitis*.

Cheilosis is highly characteristic of riboflavin deficiency. Spies found this sign in practically every case of riboflavin deficiency among children in Alabama [153]. However, cheilosis is not a pathognomonic sign. At first the dry lips, especially at the corners of the mouth, are pale; after a few days, maceration occurs and deep scars (rhagades) occur, which involve the edges of the lips, the lips themselves, and the corners of the lips, and then become crusted (Fig. 40). The entire lip may be involved later. Infection may occur. The surrounding skin becomes eczematous. Magenta-colored glossitis and changes of the cheek mucosa occur. The tip and edges of the tongue become smooth, shiny, and dry; dark red at first and later, when anemia supervenes, pale. The papillae show atrophy.

Chronic, atrophic inflammatory changes occur in the esophagus and the stomach. The secretion of hydrochloric acid is reduced. Generalized intestinal disturbances,

including both constipation and diarrhea, occur. The stool is rich in fatty acids and neutral fats. The vaginal mucosa shows pruritus and painful inflammation. Vannotti [9] emphasizes the common occurrence of chronic inflammation of the respiratory tracts.

The changes of cheilosis often extend to seborrheic dermatitis involving the eyelids, nasal folds, ear lobes, and ear canals. Eczematous lesions appear at the scrotum, penis, and vulva.

The eyes show conjunctivitis, vision is impaired, and photophobia develops.

The later stages of the disease may show a macrocytic anemia or a microcytic hypochromic anemia, as in pellagra. The marrow does not show megaloblastic erythropoiesis.

A careful dietary history is of prime importance for the diagnosis (Table 38). The diagnosis is suggested by the presence of cheilosis and conjunctivitis. Therapeutic

trial with riboflavin establishes the diagnosis. None of the symptoms or signs, however, is pathognomonic. The angular stomatitis can occur in various infections. The tongue changes resemble those of pernicious anemia, although there may be a more yellow color in the latter because of bilirubinemia. The bone marrow, of course, rules out pernicious anemia. The lesions of the esophagus resemble those of Plummer-Vinson syndrome, and iron-deficiency anemia must be ruled out. The skin changes must be distinguished from those of classical seborrheic dermatitis and moniliasis.

The diagnosis can be established by determining the degree of saturation of the body with riboflavin. An idea of this saturation can be obtained by determining the excretion of riboflavin in a 24-hour urine, in random fasting urine, and, most reliably, after intravenous loading with riboflavin [4, 8]. The normal daily excretion of riboflavin in the urine is 750 to 1250 μ [9]. Values below 200 μ are abnormal. In the loading test, 1 mg of riboflavin is injected intravenously in a fasting patient. The excretion in the next four hours must not be less than 200 μ ; normally 250–700 μ [154]. A pathological excretion indicates a deficiency state.

In rare cases, there may be acute convulsions, collapse, and death. In general, however, the prognosis with proper therapy is excellent.

The signs are relieved in a few days on an oral dose of 15 mg of riboflavin daily. If intestinal disturbances are present, the dose is given intravenously for the first few days, and then orally. The general systemic signs remain for a few more weeks. In general, combined vitamin therapy, utilizing all the water-soluble vitamins at once, is used. The optimum maintenance dose of riboflavin is 2 mg per day (Table 39) [227, 371]. The diet must also be adequate. The vitamin is present in large amounts in milk, eggs, liver, kidney, fish, yeast, bananas, and cheese.



Fig. 40. Cheilosis in riboflavin deficiency.
(Case of Dr. Tom Spies.)

Table 38. The water-soluble vitamins in some common foods.
Milligrams of vitamins per 100 grams weight [101]

Foodstuff	Thiamin	Riboflavin	Niacin	Pantothenic acid
Meat	0.1 - 0.23	0.2 - 0.38	4 - 5	0.6 - 2.0
Liver	0.38 - 0.52	1.6 - 3.7	10 - 25	4 - 6
Human milk	0.005- 0.02	0.05 - 0.16	0.2 - 0.6	0.25
Cow's milk	0.02 - 0.04	0.10 - 0.25	0.1 - 0.5	0.28- 0.37
Egg	0.08 - 0.14	0.25 - 0.3	0.8	0.8 - 4.8
Whole wheat	0.5 - 1.0	0.18 - 0.25	3 - 8	0.5 - 1.5
Bran	0.5 - 1.0	0.6	25 - 40	2 - 3
Rye	0.24 - 0.42	0.15 - 0.20	1.3 - 2.7	1 - 2
Corn	0.30 - 0.40	0.05 - 0.20	1.0 - 3.0	0.3 - 0.8
Brewer's yeast	3.0 - 15.0	3.5 - 8.0	10.0 - 50.0	12.0 - 25.0
Green peas	0.4 - 0.8	0.16 - 0.28	0.7 - 2.1	0.38- 1.0
Green beans	0.07 - 0.25	0.20 - 0.28	0.2 - 0.6	
Soya beans	0.3 - 1.4	0.30 - 0.75	4 - 20	1.2
Spinach	0.06 - 0.22	0.16 - 0.36	0.4 - 1.7	0.12
Lettuce	0.05 - 0.1	0.05 - 0.15	0.2 - 0.3	
Carrots	0.06 - 0.07	0.05 - 0.10	0.4 - 1.5	0.05- 0.25
Potatoes	0.09 - 0.18	0.03 - 0.04	1.2 - 1.3	0.2 - 0.7
Tomatoes	0.06 - 0.12	0.04 - 0.05	0.3 - 0.6	0.1 - 0.4
Cabbage	0.1 - 0.2	0.05 - 0.10	0.1 - 0.4	0.1 - 1.4
Apples	0.001- 0.04	0.004- 0.02	0.09- 0.5	0.0 - 0.006
Pears	0.03 - 0.04	0.02 - 0.12	0.2 - 0.3	0.03- 0.3
Plums	0.01 - 0.05	0.02 - 0.1		0.03- 0.3
Red currants	0.06 - 0.1	0.01 - 0.02		
Black currants	0.02 - 0.08	0.01 - 0.02		
Bananas	0.05 - 0.16	0.05 - 0.075	0.3 - 0.6	0.18

Table 39. The daily vitamin B complex requirement, according to the Committee on Food and Nutrition, National Research Council [227]

	Age in years	Thiamin, mg	Riboflavin, mg	Nicotinic acid, mg
Child	1- 3	0.4	0.6	4
	4- 6	0.6	0.9	6
	7- 9	0.8	1.2	8
Youth	10-12	1.2	1.8	12
	13-15	1.5	2.0	15
	16-20	1.8	2.5	17
		1.2	1.8	12
Man resting		1.5	1.8	15
light work		1.8	1.8	18
heavy work		1.8	1.8	18
Girl	10-12	1.0	1.5	10
	13-15	1.3	2.0	13
	16-20	1.0	1.8	15
Woman resting		1.0	1.5	10
light work		1.2	1.5	12
heavy work		1.2	1.5	15
pregnancy		1.5	2.5	15
lactation		1.5	3.0	15

Deficiency of Vitamin B₁ Enzymes

Physiology of Thiamin and its Enzymes

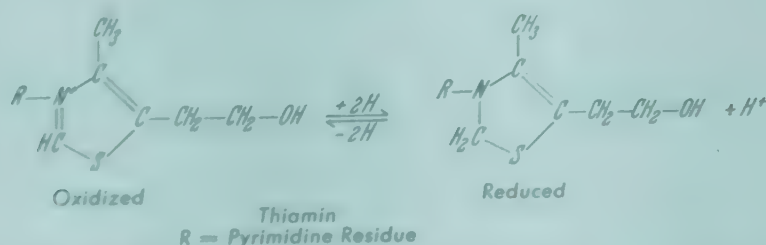
The history of the thiamin enzymes begins in 1941 with the discovery by Neuberger and Karmali [155] that preparations of yeast contain an enzyme, carboxylase, which splits carbonic acid from organic compounds. Twenty years later, Aubagen observed that this carboxylase activity of yeast is lost by washing [156], but the addition of the washings to the washed yeast restores the activity. In the process of washing, 2 essential factors pass from the yeast into the water: magnesium ions, and an organic heat-stable compound. Aubagen named the latter "cocarboxylase" and found it, not only in yeast, but also in many animal tissues. In the same year, Simola [157] showed that cocarboxylase can replace vitamin B₁ in biological tests, and that cocarboxylase is present in lower concentration in the tissues of animals with beri-beri than in the tissues of normal animals. These observations demonstrated the relationships among cocarboxylase, vitamin B₁, and beri-beri. Vitamin B₁ or thiamin is the active group of carboxylase. A deficiency of vitamin B₁ leads to the development of beri-beri.

Thiamin was crystallized in 1926 by Jansen and Donath [158]. Its structural formula and its synthesis were detailed in 1936 by Williams [159], Grewe [160], Andersag and Westphal [161], and Todd and Bergel [162]. In 1937, Lohmann and Schuster [163] described the crystallization and structure of cocarboxylase, the pyrophosphate ester of thiamin.

Carboxylase is ubiquitous in nature, splitting CO₂ from many organic compounds. The thiamin carboxylases, as far as known today, split only the α -ketocarboxylic acids into the corresponding aldehyde and carbonic acid. In some substrates, in addition to thiamin pyrophosphate, a second organic cofactor, α -lipoic acid, is also involved. Besides the thiamin carboxylases, other carboxylases also occur naturally, and act without vitamin B₁. Some of these other carboxylases require vitamin B₆ (pyridoxal) as coenzyme; others, manganese.

The chemistry of the apo-enzymes of the carboxylases is poorly understood. Green et al [164] and Kubowitz and Lüttgens [165] succeeded in separating the apo-enzymes of yeast pyruvate carboxylases from the coenzymes, and were able to purify the material. However, the apo-enzyme could not be crystallized. The apo-enzymes are assumed to be pure proteins and thus underlie protein metabolic processes of the body.

Vitamin B₁ or thiamin is an organic base which is easily soluble in water. The molecule consists of 2 heterocyclic rings, one a pyrimidine derivative and the other a thiazol, which are bound to one another by a methylene bridge. The thiazol ring undergoes reversible reduction with hydrosulfite [167].

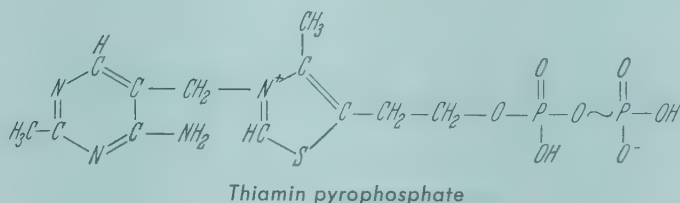


The significance of this reaction in vivo is not known. Through oxidation, two molecules of thiamin can split off the thiazol ring and then unite to form a disulfide. The disulfide can be reduced to thiamin by means of cysteine or glutathione. Heating

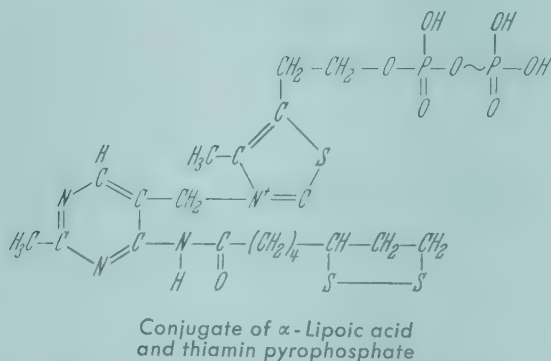
or oxidation of the disulfide leads to the yellow thiochrome, which can be measured quantitatively by its blue fluorescence [1]. In alkaline solution, the thiazol ring opens up only to close again when neutrality is reached. During the opening of the ring, a sulfhydryl group is set free. The mechanism of action of cocarboxylases may be by means of this opening and closing of the thiazol ring [168].

Reduction of thiamin produces 2,5-dimethyl-4-aminopyrimidine and 4-methyl-5-hydroxyethyl thiazol. The two substances can be reunited to form thiamin by various animals (rats, rabbits, pigeons), if the pyrimidine moiety is made somewhat more reactive by introducing an amino-methyl group or a hydroxymethyl group in place of the simple methyl group [8]. The unmodified reduction products themselves cannot be re-united to thiamin by the animal body. Thiamin is the only thiazol-containing metabolic substance known which is absolutely essential for life. Protozoa, fungi, bacteria, and most plants can synthesize both rings, and can thus synthesize the vitamin itself. The vitamin occurs ubiquitously and is necessary for all sorts of living functions. In the human body, the molecule is split by an enzyme, thiaminase, into the 2 component rings; the pyrimidine portion is excreted in the urine as pyramine, and the degraded thiazol ring as sulfate and neutral sulfur.

The coenzyme of carboxylase, thiamin pyrophosphate, has the same chemical properties as many other coenzymes: it is thermostable, dialyzable, and contains a pyrophosphate group, which is a high-energy phosphate compound. Corresponding

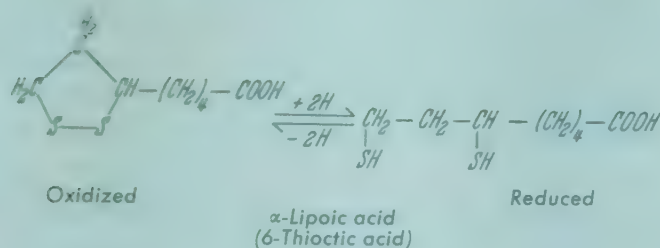


to the disulfide of thiamin there is a disulfide of cocarboxylase; this, however, has no biological activity. In addition to the pyrophosphate derivative of thiamin, other compounds known are thiamin monophosphate, thiamin triphosphate, and lipothiamin pyrophosphate. Thiamin monophosphate and thiamin triphosphate have been synthesized. Thiamin monophosphate, like thiamin itself, has no coenzyme action. Thiamin triphosphate can substitute for cocarboxylase in yeast, but has not yet been found in biological material. Lipothiamin pyrophosphate is postulated to be the coenzyme of various carboxylases [170]. This substance is a conjugate of α -lipoic acid and thiamin phosphate:



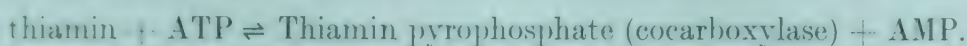
This α -lipoic acid has a short history since its discovery in 1941 as a growth factor for protozoa [169]. Various names have been given to it, including protogen A, pyru-

rate oxidizing factor, 6-thioctic acid, etc. It is found extensively in nature, in animal liver, in bacteria, in yeast, and in various plants. The free acid is soluble in fat; the bound form is soluble in water. The substance has recently been crystallized and its structure determined. The 6,8-dithio-octanoic acid (6,8-dithiocaproic acid) occurs as a disulfide in the form of a dimercapto or a mono-thio-acyl-mono-mercapto form:



In vivo enzymatic union of lipoic acid occurs with the amino group of thiamin to produce lipothiamin pyrophosphate. The significance of this conjugation is not clear [170, 175]. Nothing is known, too, of the catabolism, metabolism, and circulation of the compound in the body. The substance α -lipoic acid is a vitamin, but a deficiency syndrome has not been described in man.

In the body, cocarboxylase results from the reaction of ATP with thiamin [171]:



Splitting is accomplished by a pyrophosphatase at the pyrophosphate bond in the same way as in other coenzymes (DPN, TPN, FAD). Thiamin ingested in food is readily absorbed in the small intestine. Amounts in excess of 5 mg per day are broken down or are excreted unchanged in the stool. Bacterial synthesis occurs in the colon but plays no role as a source of the vitamin. In the human, whole blood contains approximately 7.5 $\gamma\%$ of thiamin (2-16 $\gamma\%$ by different methods) [6]. A small portion of this (1 $\gamma\%$) is present in the plasma as free thiamin, and the remainder is present in phosphorylated form within the blood cells. The exchange of thiamin between interstitial and intracellular fluid occurs readily in both directions. The cocarboxylase is synthesized enzymatically within the cells. Thiamin is present in all tissues: the skeletal musculature contains 50% and the liver 30% of the total body thiamin. The heart contains 200-300 $\gamma\%$; liver, brain, and kidneys 100 $\gamma\%$; muscle 50 $\gamma\%$. The free thiamin of the tissues is 1-4 $\gamma\%$. The peripheral nerves contain approximately 50 $\gamma\%$ of free and 100 $\gamma\%$ of bound thiamin [8].

Storage of thiamin does not occur. The urine regularly shows free thiamin as well as pyramine, but no cocarboxylase.

The thiamin enzymes act only on the α -keto-carboxylic acids, splitting off carbon dioxide. The active group of the enzymes is thiamin, which is probably bound to protein by means of pyrophosphate and magnesium. Kubowitz and Lüttgens [165] found that their most active preparation contained 1 gram-atom of magnesium and 1 mol of thiamin diphosphate per 25,000 grams of protein. (This is the same ratio in which alloxazine and protein occur in the yellow enzyme [172].) Langenbeck [173] hypothesizes that the primary amino group of thiamin reacts with the α -keto-carboxylic acids by the formation of a Schiff base and coupled decarboxylation. The product of decarboxylation is an aldehyde which is bound to the thiamin-pyrophosphate-enzyme complex (TPP):



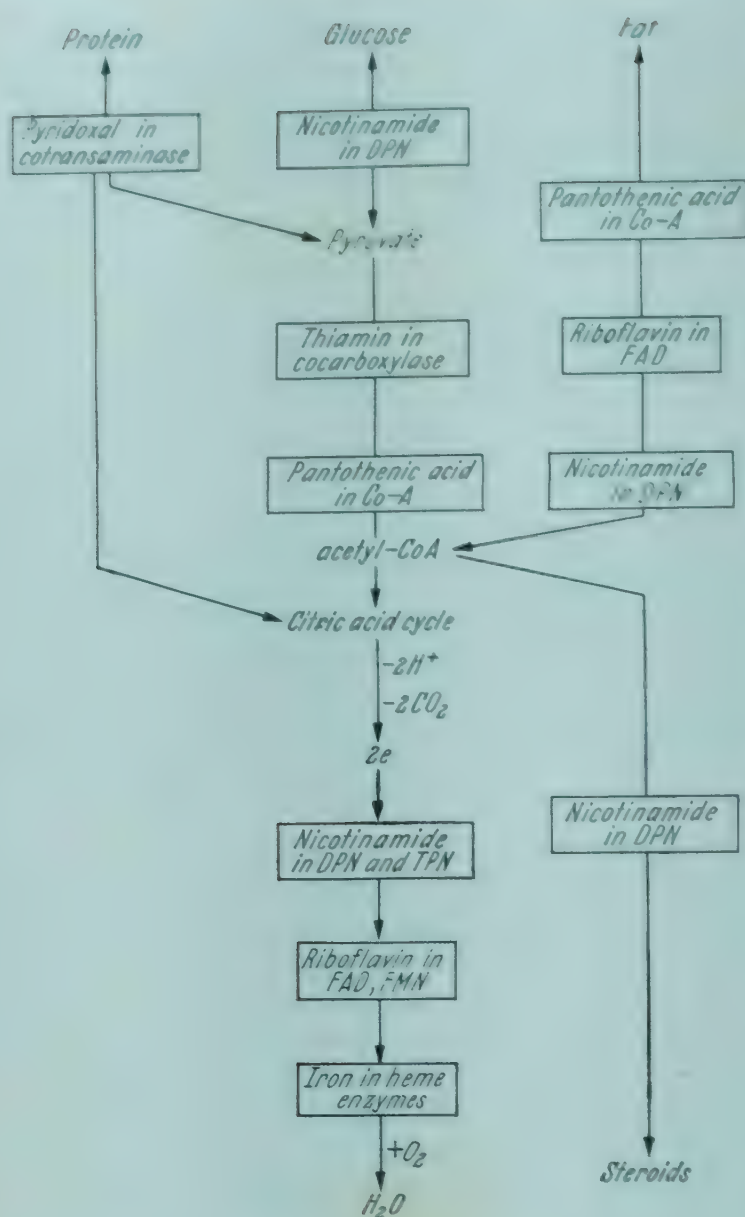


Fig. 41. The place of the vitamins in glycolysis, oxidative catabolism, and fat and steroid metabolism.

This "activated aldehyde" can undergo further reaction in various directions by hydrolysis, condensation, or oxidation of the enzyme itself or by intervention of other enzymes [170]. Thus, various intermediate substances are found as end products of decarboxylation. In yeast there is decarboxylation of pyruvate and the enzyme-TPP-aldehyde complex is hydrolyzed with the formation of free acetaldehyde and carbon dioxide. In animals there are complicated secondary reactions. Thus, the activated aldehyde is found as acyl-CoA after the oxidation of coenzyme A (CoA) by pyridine enzymes (p. 140). Acyl-CoA itself can undergo further reactions. The reaction is reversible. Apparently, in the transfer of the acylated aldehyde, α -lipoic acid also plays a role, either as the free coenzyme or in combination with thiamin pyrophosphate. These anabolic and catabolic mechanisms of the α -keto-carboxylic acids have a fundamental significance for the metabolism of the different biochemical substances (carbohydrates, fats, proteins), for their conversion into one another, and for their catabolism

with the development of energy in cellular respiration. The most important of the α -keto-carboxylic acids in metabolism are pyruvate and α -keto-glutarate. Furthermore, thiamin pyrophosphate plays a significant role as a coenzyme of transketolase for the formation of "active glycolaldehyde" in the pentose-phosphate pathway.

Pyruvate is the end product of glycolysis and is also important in the transamination of amino acids. In the presence of magnesium ions, pyruvate is decarboxylated by α -carboxylase I (which can be prepared from heart muscle of various species [174]). The reaction proceeds by way of activated acetaldehyde to produce, by oxidation, acetyl-coenzyme A. Pyruvate is thus the starting material for a number of important metabolic processes, including the synthesis of fatty acids and steroids, the formation of acetyl choline, the acetylation of certain toxic metabolic products (detoxification), and the formation of citrate and oxalacetate (p. 142).

The second substance, α -ketoglutarate, is decarboxylated by carboxylase II in the presence of magnesium ions; succinic acid semialdehyde is formed; and oxidation yields succinyl-coenzyme A [175]. This coenzyme A derivative is a component of the citric acid cycle and is converted to fumarate, but at the same time is in equilibrium with acetoacetate and therefore with the fatty acids. The α -keto glutarate is a degradation product of citrate and at the same time an acceptor of ammonia, forming glutamate by simultaneous reduction or transamination. Glutamate is a component of glutathione, of folic acid, and of many proteins; and is an important ammonia buffer.

There are close quantitative relationships between the metabolism of pyruvate and α -keto-glutarate on the one hand, and the metabolism of thiamin on the other. Since the turnover of the two substances depends on the rate of ingestion of carbohydrates, proteins, and fats and their utilization, storage, or oxidation within the body, these factors also affect the requirement of the body for thiamin. The thiamin requirement of the human body on a predominantly carbohydrate diet is 1 molecule of thiamine per 10^6 molecules of glucose (20 γ thiamin per 110 grams of glucose).

In the oxidation of fat, the body spares thiamin, for in this process pyruvate is not involved [46]. Fatty diets thus spare the vitamin, while carbohydrate-rich diets increase the requirement for thiamin. Protein-rich diets also spare vitamin B₁, although not to the same degree as fat, for a portion of the amino acids undergoes transamination into pyruvate.

There is also a connection between the action of thiamin and that of insulin. Thiamin deficiency leads to resistance to insulin [176, 177]. The administration of vitamin B₁ can often eliminate glycosuria and restore hepatic glycogen formation to normal.

The connection between vitamin B₁ and thyroid hormone must also be mentioned. Hyperthyroidism leads to an increased requirement of substrates for the increased amount of oxidation in the body. The substrates are produced from glycogen by way of pyruvate, with the aid of cocarboxylases in the citric acid cycle. In the citric acid cycle itself, α -keto-glutarate is steadily decarboxylated. It is therefore easily understood that, when hyperthyroidism occurs and leads to an increased use of substrate, there is also an increased need for thiamin [8]. If the amount of cocarboxylase or thiamin present is not sufficient, the action of the thyroid with respect to the increased oxygen consumption may be inadequate even though there is overproduction of hormone, for there is insufficient synthesis of substrate for the processes of oxidation.

The importance of optimal amounts of thiamin can be demonstrated in various physiological functions. Thiamin improves the utilization of carbohydrate by the working muscle [178]. The vitamin has an important growth function, a fact which was formerly used to measure it biologically. Further discussion of the functions of thiamin appears below in the section on pathology of the thiamin enzymes.

The daily requirement of the body for vitamin B₁ is determined by the quantitative relationships between carbohydrate metabolism and the vitamin. The vitamin content of the body is best tested by a loading test (page 137). The vitamin and its coenzyme can be determined chemically or microbiologically. The microbiological method utilizes increased growth of test organisms [179, 4]. The chemical method depends on the demonstration of thiochrome [4, 8].

With the knowledge of the structure of thiamin, studies were undertaken to determine the part it plays in enzyme physiology. Alteration of the structure to form structural analogs, by introduction of side chains or exchange of functional groups, reduces the biological activity or may even produce an antagonistic substance (inhibitor or antivitamin). (The mechanism of action of the antivitamins depends on com-

petition between vitamin and antivitamin for the enzyme, the competition depending on the similarity of the structures of the two substances. The antivitamin replaces the vitamin from its position at the surface of the enzyme and thus changes the function of the enzyme. A special case of such replacement is competitive inhibition.)

The following substances have such anti-thiamin activity: hydroxythiamin, produced by replacing the 4'-amino group of the pyrimidine ring by a hydroxyl; the substance produced by replacing the 2'-methyl group of the pyrimidine by a N'-propyl residue; and pyrithiamin and neopyrithiamin, produced by replacing the thiazol ring by pyridine.

Hydroxythiamin is converted within the cells to hydroxythiamin pyrophosphate in a manner analogous to the conversion of thiamin to thiamin pyrophosphate (p. 129). The resulting substance is a "false cocarboxylase," whose active group is altered with respect to the 4' position of the pyridine ring. According to Langenbeck, the 4' position of carboxylase contains an amino group, so that a Schiff base can be formed (p. 129); in addition, α -lipoic acid can be bound by the amino group. Neither reaction can occur in the false cocarboxylase. Nonetheless, the cocarboxylase analog reacts with the apo-enzyme and replaces natural cocarboxylase from its position. Injection of hydroxythiamin into a mouse leads to death after two weeks, for the substance leads in vivo, as well as in vitro, to the synthesis of a false, lethal cocarboxylase. Peters describes a similar, lethal type of synthesis in poisoning with fluoracetic acid [66].

Pyrithiamin has an anti-thiamin action whose nature and mechanism are not yet known. The differences between the mechanisms of action of hydroxythiamin and pyrithiamin show up in the respective clinical pictures. Pyrithiamin and neopyrithiamin produce severe polyneuritis and death, but polyneuritis does not occur with hydroxythiamin [180]. In both pyrithiamin and neopyrithiamin the thiazol ring is replaced; since only these two substances produce polyneuritis, polyneuritis may reasonably be ascribed to the change in the thiazol ring.

Pathology of Thiamin Deficiency

Beri-beri was first described by the Chinese in the seventh century. Almost 1,000 years later, when polished rice was introduced as a staple of the diet, beri-beri appeared in Tokyo. In 1627, Bontius in Batavia first used the term "beri-beri," derived from the Hindustani word "beri," which means sheep (referring to the sheep-like gait of the patients). In 1882, Baelz [181] described beri-beri as an endemic polyneuritis. The Japanese physician Takaki made observations on the relationship of diet to beri-beri [182]. By substituting meat (i. e., protein) and vegetables for rice in the diet of Japanese sailors, he reduced the morbidity from 23% to 0.5%. The Dutch East Indian physician Eijkman [183] showed the connection between polished rice and beri-beri. He observed that laboratory chickens fed with the food discarded from hospital kitchens developed a polyneuritis which resembled human beri-beri; this polyneuritis disappeared when the diet was changed or when rice husks were added to the polished rice diet. Vordemann [184], in studies on Javanese prisoners, showed that prisoners fed on a diet of polished white rice developed beri-beri in an incidence of 1:39, while in prisoners fed unpolished rice the incidence was 1:10,725 individuals. These observations allowed control of the disorder by dietary means [185]. Even today, however, beri-beri is very common in the Far East. In 1911, Funk [186] isolated a nitrogen-containing base which had anti-beri-beri activity, to which he gave

the original name "vitamin." The particular vitamin involved in beri-beri was shown to be vitamin B₁ or thiamin, but it was almost 20 years before its composition was determined. Peters [187] then showed, in experiments in polyneuritic pigeons, that thiamin plays a fundamental role in the metabolism of pyruvate.

The pigeon is the classical experimental animal for the study of beri-beri. Some 20 to 30 days after the beginning of a thiamin-free diet, the pigeon loses its appetite and becomes weak and apathetic. The weight falls, and the body temperature goes down. Digestive disturbances appear. Finally, the characteristic cardiac and neurological signs appear, including tachycardia, marked changes of the electrocardiogram, and flaccid and spastic paralyses. The gait becomes uncertain and the pigeon tends to fall to one side and supports itself with its wings. Opisthotonos and generalized muscular spasms, especially on touch, develop. Ultimately, there is paralysis of the muscles of respiration and death. The same general picture is seen in other laboratory animals, although the predominating symptom may vary from animal to animal. In rats, the first sign is loss of weight, and inhibition of this weight loss has been used as a biological test for vitamin B₁. Later, the rats show loss of appetite, reduced body temperature, and bradycardia. In chickens, abnormal gait and opisthotonos occur [183].

Peters studied the oxygen utilization of brain slices from normal and beri-beri pigeons and found that pyruvate is oxidized more slowly by the diseased brain slices than by normal brain. Addition of vitamin B₁ in vitro eliminates the abnormality. The reduction of oxygen utilization in pyruvate oxidation was proportional to the brain content of thiamin or cocarboxylase.

If the amount of thiamin or cocarboxylase in various tissues is determined as the clinical picture of beri-beri develops in experimental animals, it is found that there is a steady decrease with the progression of symptoms and signs. The concentration falls first in the muscles, later in the brain and heart. At the same time, the excretion of the vitamin in the urine is reduced to a minimum. To the same degree that there is a fall of the coenzyme content of the tissues, there is an increase of those metabolic products whose catabolism cannot be accomplished because of the coenzyme deficiency, especially pyruvate and α -ketoglutarate. These substances increase in the tissues, blood, cerebrospinal fluid, urine, and stool. The increase is a sensitive measure of the degree of thiamin deficiency, and is followed by a profound change of cellular metabolic equilibrium.

Lactate also increases, because of enzymic reduction of pyruvate to lactate. When the convulsive stage of the disease occurs, there is also an increase in the blood sugar. As a result of enzymic block of catabolism of pyruvate, there is insufficient production of the degradation product of pyruvate, acetyl-CoA. There is reduced concentration of all products containing this material – metabolites of the fatty acids (acetoacetic acid, etc.), of the sterols and steroids (adrenal cortex, testes, liver), and of the acetylation products such as acetylcholine (nervous system) and acetylsulfonamides (detoxification reactions). The citric acid cycle is damaged by thiamin deficiency at the α -ketoglutarate step. The synthesis of citrate is greatly reduced, as indicated by the reduced excretion of citrate in the urine, while the excretion of α -ketoglutarate rises. The cycle supplies approximately 75% of the intermediate products used for oxidation in cellular respiration. The result of deficiency of these intermediate materials is a fall of oxygen utilization and impaired energy-production by cellular respiration. There is reduced conversion of inorganic phosphate to high-energy phosphate, and the concentration of inorganic phosphate in the blood and muscles rises. There is a fall of the body ATP, with resulting impairment of the energy-requiring processes of

the cell. Finally, there is a fall of body temperature. The increase in pyruvate and α -ketoglutarate is most marked in those tissues which show particular sensitivity to oxygen and especially high requirements for carbohydrate and thiamin, i. e., the heart and the nervous system. This is reflected in the pyruvate concentration of different veins; for example, the blood of the coronary vein as compared to the vein of an extremity shows a higher content of pyruvate [188].

The clinical response of the tissues to reduction of energy supply as a result of deficiency of oxidizable substrate is always the same. Peters [66] showed that pigeons react with convulsions when there is reduced amount of substrate as a result of experimental inhibition of the citric acid cycle at any point (Fig. 29, p. 92). The catabolism of pyruvate is impaired when there is deficiency of thiamin or poisoning with fluoracetic acid or arsenic. (Arsenous acid binds α -lipoic acid; fluoracetic acid produces the lethal fluoroethtric acid within the cell.) Hypoglycemic convulsions are also precipitated by deficiency of oxidizable substrate. In general, human beri-beri shows no differences from experimental beri-beri in animals. The chief symptomatology involves either the nervous system or the heart.

Clinical Beri-Beri

Beri-beri is still endemic in the Far East and can be related to the dietary habits of the populations of India, Indonesia, the Philippine Islands, China, and Japan. In 1947, there were 132 deaths per 100,000 population in the Philippines, the same mortality as that of tuberculosis [189]. The disorder also tends to occur in Europe and North America, for the usual diet just covers the daily thiamin requirement [8]. Sporadic cases are prevalent in industrial centers and in cities and affect all social classes, in contrast to avitaminoses A, C, and D, which are most common among the poorer classes. The disorder is endemic in many places, as in Hungary in 1935. However the disease is common only in those countries in which rice is the chief constituent of the diet, especially when polishing removes the rice hull with its vitamin content. Natives who do not cultivate rice are usually not affected. The incidence of beri-beri is increased during pregnancy and lactation, in breast fed infants, and in young men. **Women are generally affected less often than men.**

Several mechanisms may be responsible for the development of beri-beri. The intake of thiamin may be reduced, as in unbalanced diets, breast feeding in deficient mothers, dietary idiosyncrasies, disorders of the stomach, heart, or kidneys, and diets of mentally ill patients and inhabitants of institutions. Impaired absorption of the vitamin occurs in chronic intestinal disorders, enterocolitis, and malabsorption syndrome. Deficient incorporation of thiamin in the coenzyme and deficient synthesis of the apo-enzyme occur in protein-deficient diets and in hepatic diseases. Finally, the requirements for thiamin may be increased in various disorders and thus predispose to hypovitaminosis if the increased requirement is not met. Such states include high-carbohydrate diets, increased use of alcohol, severe muscular effort in work and sports, pregnancy (especially if there is vomiting), lactation, growth, high environmental temperature (as in the tropics), diabetes mellitus, chronic fever such as **malaria, and hyperthyroidism.**

Classical beri-beri is characterized by *cardiovascular* manifestations, *edema*, and *polyneuritis*. In northern latitudes, the "dry" form of beri-beri is most common. The chief signs occur in the nervous system and the heart, with slight edema of the lower extremities. In contrast to this occidental beri-beri, there is the oriental or tropical or

"wet" beri-beri, whose manifestations are chiefly cardiovascular [190]. Wernicke's encephalopathy has recently been shown to be due to thiamin deficiency by Jolly [191]. Beri-beri heart disease, encephalopathy, and acute beri-beri are relatively uncommon in Europe where nervous system manifestations are most prominent.

The signs of beri-beri may remain latent for months and become manifest only after the addition of an extra burden such as pregnancy or bodily effort. They may also occur as secondary manifestations in other diseases. In volunteer subjects on a thiamin-deficient diet, subjective symptoms occur after only three days and objective signs after four days on the diet.

Symptoms include generalized weakness, especially in the legs on walking; depression and irritability; poor memory, insomnia, fatigue, muscle cramps; and diarrhea. The appetite is lost, the weight falls, and palpitation, dyspnea, and shock ultimately occur. Amenorrhea is present. There is often slight swelling of the ankles.

These prodromal symptoms pass either gradually or suddenly into the overt disease.

In dry beri-beri, the chief signs involve the nervous system. Even the old Chinese writings mention paralysis of the extremities. The clinical picture includes gradually progressive symmetrical polyneuritis, which usually begins with sensory disturbances (hyperesthesiae, paresthesiae, and anesthesia of the lower extremities). The thighs are especially sensitive. The tendon reflexes are at first exaggerated, later reduced, and finally absent. As the disease progresses, the muscles of the legs atrophy, although slight edema may mask the atrophy (Fig. 42). The patient cannot stand up from a squatting position without help, a paralytic gait develops, and flaccid paralysis ultimately occurs. At the same time, the upper extremities become involved, so that the use of the hands and arms is lost, and the patient becomes helpless. Paralysis of the extremities may result in contractures and deformities. Ultimately, the vagus and the cranial nerves are affected. Ophthalmitis, diplopia, hoarseness, aphonia, diaphragmatic paralysis, and incontinence of stool and urine occur. In contrast to pellagra, the central nervous system is usually not involved, except in those cases in which Wernicke's syndrome occurs.

In cardiovascular ("wet") beri-beri, there are first minor complaints of palpitation, slight dyspnea on effort, and fleeting edema of the ankles. The heart rate is slow during rest and very rapid on effort. The electrocardiogram shows flattening or inversion of the T wave, reduced voltage, prolongation of the QRS complex, and rarer conduction disturbances [192, 193, 194, 195]. As the disease progresses, cardiac and vascular signs become more marked. Early in the disease, either the vascular or the cardiac signs are prominent; later, a vicious circle is set up.

There is increase of the minute volume of the heart (cardiac output), with resulting changes in the peripheral circulation. The arterioles and arteriolar capillaries are



Fig. 42. Pitting edema of the ankles in beri-beri. (Case of Dr. Tom Spies.)

widened, and the peripheral resistance is reduced. The circulation time is shortened and the blood volume increased. The venous pressure rises. The pulse is rapid and high. As cardiac involvement progresses, right heart failure occurs. Edema occurs at the ankles (Fig. 12), scrotum, in the serosal cavities, and in the joint spaces. The edema fluid shows a high content of sodium chloride and a low protein content. Beri-beri edema is primarily the result of vascular damage and is not related to the kidneys. The prominent edema gives the name "wet" to this form of beri-beri.

In many patients, cardiac decompensation is an early event because of primary avitaminotic damage of cardiac muscle. Wenekebach [190] observed this in the Orient chiefly as right heart failure, but in western countries left heart failure predominates. **Wenekebach's cases frequently died of acute right-sided failure.**

Fulminating beri-beri is an especially acute form of cardiovascular beri-beri. The patient shows marked anxiety, precordial pain, and acute dilatation of the heart with increased venous pressure, reduced arterial pressure, and anuria. Disturbances of the intestine are marked and include vomiting and diarrhea, and death is due to cardiac failure.

Wernicke's "polioencephalitis hemorrhagica superior" is a form of cerebral beri-beri. The initial symptoms are anorexia, vomiting, nystagmus, and psychic disturbances. Cranial nerve disturbances, especially ophthalmoplegia, occur later, together with pyramidal signs and bladder and rectal disturbances. The protein content, colloidal gold reaction, cell count, and glucose of the cerebrospinal fluid are all normal. The symptoms and signs often come on in association with diarrhea, and are usually seen in alcoholics. The syndrome may also be seen in infants. The pathogenesis is assumed to be acute thiamin deficiency.

Beri-beri in infants affects almost exclusively infants on the breast in the first 3 months of life. The chief signs are cardiac. "Aphonic" and "pseudomeningitic" forms have also been described. Edema occurs in the face and lower extremities, there are cardiac dilatation and circulatory disturbances, and vomiting and diarrhea result in apathy. **The children die of pulmonary edema.**

The diagnosis of beri-beri is suggested when the classical signs of cardiovascular abnormalities, polyneuritis, and edema are marked. The diagnosis can be established by demonstrating thiamin deficiency by a load test. Beri-beri must be distinguished from other causes of cardiac and renal disease and from other peripheral neuritides. The dietary history is, of course, of prime importance (Table 38). The patient often admits to a diet rich in carbohydrates (rice, corn, wheat) and poor in vitamins and proteins. Modern diets used for the treatment of hypertension and glomerulonephritis are usually vitamin-deficient [196]. Other disorders and deficiencies of other vitamins may confuse the picture (e. g., pellagra) and make diagnosis difficult or even impossible. In the presence of malnutrition, pure nutritional edema must be differentiated. The urine shows neither casts nor protein. As the disease progresses and cardiac signs occur, the kidneys become engorged and albuminuria occurs. The discrepancy between the frank edema and the paucity of urinary findings differentiates beri-beri from renal disease, especially nephrosis. The effects of therapy with thiamin differentiate beri-beri from neuritides due to other causes (infection, vertebral compression, tabes). In doubtful cases, the administration of 50 mg of thiamin per day intravenously for several days will allow diagnosis. The cardiac manifestations are resistant to the use of digitalis.

There is always an elevation of pyruvate in the serum in cases of beri-beri. The normal concentration (by the enzymic-optical method of determination) is 0.4 mg %.

[197]; pathologically, this can be elevated from 3 to 8 times, up to $3 \log_{10}$. (The determination of pyruvate in the serum employs a specific lactate dehydrogenase and reduced nicotinic in an enzyme-optical method. It is simple and extremely specific [197]. The older methods of determining the so-called bisulfite-binding substance and other chemical methods of determining pyruvate also measure other α -keto-carboxylic acids. They are not specific and not reliable). An elevation of pyruvate in the serum also occurs in diabetes mellitus, cardiac and renal diseases, bodily effort, after eating, and in psychic disturbances.

The quantitative relationships among glucose, lactate, pyruvate and co-carboxylase can be studied by loading tests which measure the serum pyruvate after the administration of glucose. The serum value indicates the rate of catabolism. Normally, following the administration of glucose, there is a rise in the serum pyruvate which is maximal after 1 hour and returns to normal after 3 hours [198]. In thiamin deficiency, the curve is prolonged and elevated.

The loading test is already positive when the tests for vitamin saturation still show only a latent deficiency. The steady-state concentrations of pyruvate, lactate, and glucose, on the other hand, become elevated only after the saturation and loading tests are frankly abnormal. Horwitt and Kreisler [199] devised a carbohydrate metabolism index (CMI) to give the relationship among lactate (L), pyruvate (P), and glucose (G) in $\text{mg}\%$ in blood obtained 5 minutes after the completion of a mild stair-climbing test applied 60 minutes after the oral administration of 1.8 gm of glucose/kg body weight. Under normal conditions, the CMI is 15 or less:

$$\text{CMI} = \frac{\text{L} + 15 \text{ P}}{2} - \frac{\text{G}}{10}$$

The concentrations of free and bound thiamin in the blood show large variations. The determination of serum thiamin is therefore of no practical value, and better insight into the thiamin content of the body is attained by determining the degree of saturation of the body. Optimal saturation corresponds to optimal function and, since there is no real storage of thiamin in the body, the excretion of thiamin in the urine, especially after a test dose, is an excellent measure of the degree of body saturation. The simple determination of urinary thiamin shows wide variations and is therefore only of gross value in estimating the body content of the vitamin. An average daily excretion of less than 100 γ of thiamin is a crude indication of thiamin deficiency. Many saturation tests have been suggested. In general, the administration of 1 mg of thiamin intramuscularly or intravenously results in the appearance in a 24 hour urine of approximately 20% of the test dose. When the excretion is only 10%, there is latent thiamin deficiency; when it is 5%, the deficiency is absolute [200]. A pathological result thus indicates a state of deficiency. Hypovitaminoses are frequent in neuropathies, diarrheas, hyperthyroidism, and cardiac decompensation, unless the vitamins are administered therapeutically.

The prognosis depends on the stage of the disease and, especially, on the amount of cardiac damage. Infants are especially susceptible. In one series, follow up of 100 cured patients showed no cardiac residua [192].

Therapy of beri-beri with thiamin rapidly leads to cure [372]. The initial dose may be 100 mg of thiamin two or three times a day, preferably during meals, but larger doses may be used. If intestinal disturbances are present, the vitamin may be given intravenously. In addition to thiamin itself, other vitamins should be given in the form of multivitamin preparations, yeast, or crude liver; and, in addition, protein

must be given. The daily thiamin requirement during health depends on numerous factors, among which the type of diet and physical work are the most important. In general, the modern diet, with its purified types of food, contains only a small amount of vitamins as compared with the natural diets of past years. Thus, the daily diet of the English farmer of the 15th century contained an average of 4 to 5 mg of thiamin, while that of the 19th century contained only 1.0 mg [201]. During illness, the daily requirement also depends on other factors which increase the metabolic rate of the body. The requirement is doubled during fever and in patients with malignant tumors or hyperthyroidism. Various calculations of the daily thiamin requirement have been made, utilizing the excretion of pyruvate in the urine, the saturation tests, and the pyruvate curve after loading with glucose. According to Williams and Spies [202] and Cowgill [203], the amount of vitamin taken in is adequate when Q in the following formula is greater than 0.3:

$$Q = \frac{\text{thiamin intake per day in } \gamma}{\text{total daily calories exclusive of fat.}}$$

The maintenance dose depends on general physiological principles, including optimal growth in infancy and childhood, and optimal health in adulthood. The thiamin requirement of a child aged 1 to 3 years is 0.4 mg per day; that of a man performing heavy work, 1.8 mg per day (Table 39) [372].

Pantothenic Acid Enzymes

Physiology

The pantothenic acid enzymes were discovered in 1945 by Lipmann [204], who found that the enzymatic acetylation of sulfanilamide by hog liver required an activating substance. Lipmann named the dialyzable, heat-stable cofactor the "co-enzyme of acetylation," or CoA. At approximately the same time, Nachmansohn and Berman [205], Feldberg and Mann [206], and Lipton and Barron [207] reported that an activating substance was also necessary for the acetylation of choline to acetylcholine. Lipmann and Kaplan [208] then showed that CoA was identical with the activator of choline acetylase and is actually the coenzyme responsible for the acetylation of a large number of other substrates. In 1947, Lipmann showed that CoA is a derivative of pantothenic acid [209]. The apoenzyme of acetylation is a protein. In 1951, Lynen, Reichert, and Rueff [210] found that CoA can be converted enzymatically to acetyl-CoA, and showed that this substance is identical with so-called "activated acetic acid," whose existence had long been postulated. Acetyl-CoA can transfer its acetyl residue to acetyl acceptors such as choline, sulfanilamide, and other substances. CoA is therefore a coenzyme which transfers a chemical group, the acetyl group. It was later found, however, that the action of CoA is not limited to the acetyl group alone, but also includes all acyl residues. Thus, for example, fatty acids and their intermediate products of β -oxidation undergo anabolism and catabolism not as the free acids themselves, but as CoA derivatives. Knoop's theory has thus undergone expansion, and Lynen [46] has reformulated it in the form of a fatty acid cycle (Fig. 43). Lynen also showed that acyl-CoA should be considered as acyl mercaptan. The acyl mercaptan bond is a high-energy bond [21], and its energy content is the same as that of the high-energy phosphate bond.

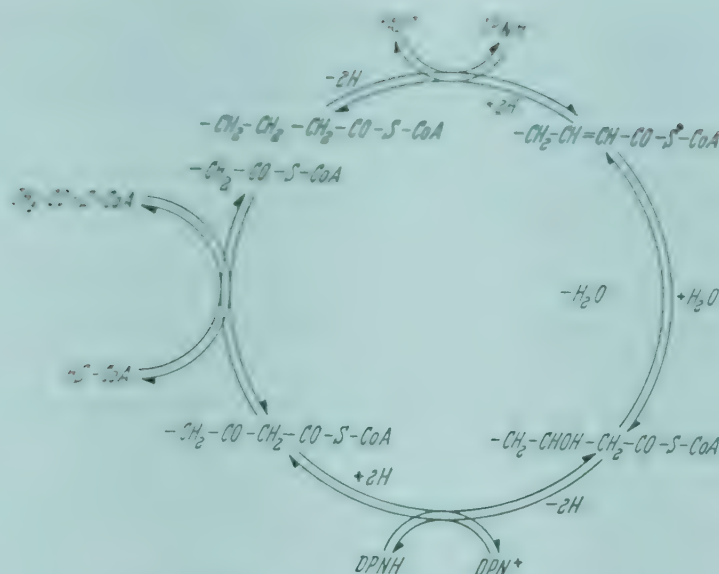
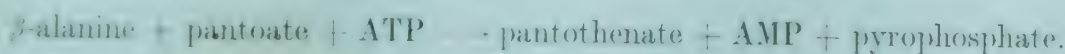


Fig. 43. The Knoop-Lynen Fatty Acid Cycle.

From the structural point of view, CoA belongs, like the coenzymes of the pyridine and the flavin enzymes, to the nucleotides. Its structure consists of an adenylic acid portion and a pantothenic acid portion [211] (Fig. 44). Pantothenic acid owes its name to its ubiquitous distribution. It is an important growth factor for many microorganisms and an important vitamin for higher forms of life. Chemically, pantothenic acid is a dipeptide of β -alanine and a substituted butyric acid. It is synthesized in the leaves of green plants and in many microorganisms from β -alanine and pantoate [219]. The synthesis requires ATP [213, 213 a]:



The source of pantoic acid is unknown; the β -alanine is probably the result of decarboxylation of aspartate. In animals, pantothenate is probably converted in the

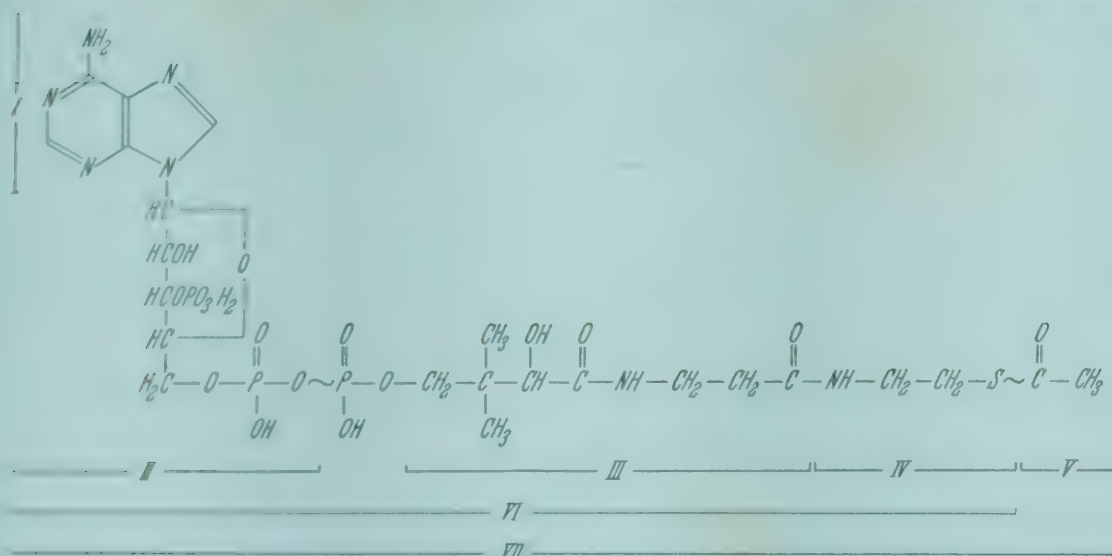


Fig. 44. The structural formula of acetyl-coenzyme A.

I. Adenine. II. Diphospho-adenosine. III. Pantothenic acid. IV. Thio-ethanolamine (β -mercapto-ethanolamine). V. Acetyl residue. VI. Coenzyme A. VII. Acetyl-coenzyme A.
~ S. High-energy sulfur bond.

presence of ATP and cystein to pantothenycysteine and then to pantethein. The synthesis of CoA from pantethein and adenylic acid components has not yet been accomplished [214, 211 a]. This synthesis takes place in several steps each requiring the presence of ATP. The degradation of the coenzyme occurs in a manner similar to that of the pyridine and the flavin enzymes, by splitting by means of a nucleotido-pyrophosphatase to adenylic acid and 4'-phosphatepantethein and finally pantothenate.

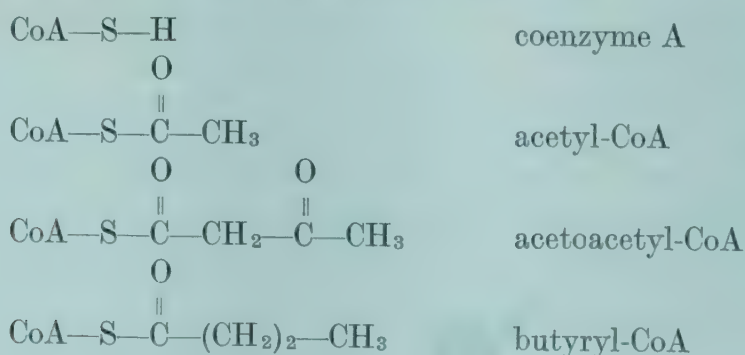
There are few quantitative data concerning the distribution of pantothenic acid in the body (Table 40). The vitamin is taken in chiefly in bound form. It is partly set

Table 40. The pantothenic acid content of rat tissues (mg% of fresh weight) following normal diets and diets deficient in pantothenic acid. From [8]

	Normal	Pantothenic acid deficiency
Liver	11.8–17.7	6.3–6.9
Kidneys	10.2–16.0	7.1–9.0
Heart	4.8– 5.0	2.4–3.4
Skeletal muscle	3.3– 4.8	1.9–3.2
Brain	5.7	4.2

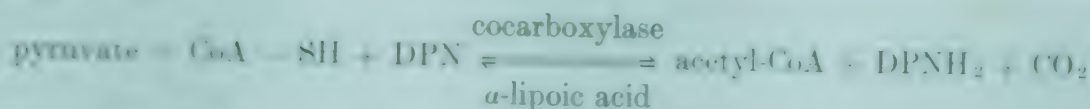
free in the intestinal tract by hydrolytic enzymes and is absorbed in phosphorylated or still bound form through the intestinal wall. The plasma contains virtually no free pantothenic acid. When the intake is normal, approximately 3 mg of pantothenic acid are found in the urine in 24 hours. The clearance of pantothenic acid is 300 to 600 cc/min [215]. The free vitamin is converted in all body cells into CoA, and all the pantothenic acid within the cells is present as CoA. The circulating plasma contains no CoA; the red cells contain 210 to 280 $\gamma\%$ [216]. The human requirement for pantothenic acid is probably completely covered by the synthesis of pantothenic acid by the intestinal flora, especially *E. coli*.

The active group of CoA is the free sulfhydryl group of pantethein. Lynen and his coworkers [46] showed that acyl groups – for example, the carboxyl group of acetic acid or other carboxylic acids – are bound enzymatically by way of the SH-group in the manner of an acyl-mercaptan. The following examples illustrate this concept:

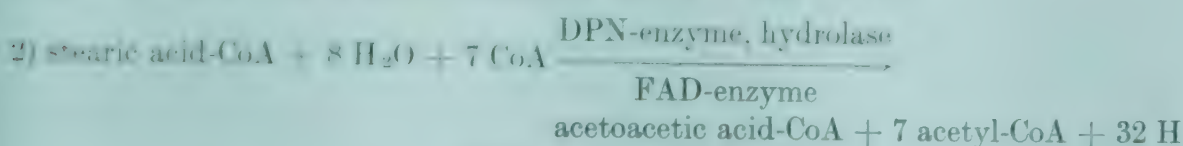
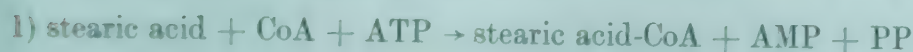


The formation of the high-energy acyl mercaptan bond activates the poorly-reacting carboxylic acids so that they are capable of reacting with many different substances within the cells. The physiological function of CoA consists therefore of activation of carboxylic acids with the formation of acyl-CoA derivatives, and thus of the transfer of acyl groups in metabolic processes. The mechanism of formation of the acyl derivatives depends on the source and type of acyl groups.

The most important of the various acyl derivatives in metabolism is acetyl-CoA (activated acetic acid), which can be formed from carbohydrates or fatty acids. Acetyl-CoA derived from carbohydrates is formed by oxidative decarboxylation of pyruvate, by way of a C_2 -substance bound to α -lipoic acid which is transferred to CoA (p. 128):



The free fatty acids are converted into the corresponding CoA derivatives by means of ATP and can then undergo degradation through the Knoop-Lynen cycle to acetoacetyl-CoA (Figure 43). The acetoacetic acid derivative is acted upon by thiolase in the presence of a molecule of CoA to form two molecules of acetyl-CoA, as follows:



Summing, $\text{stearic acid} + 9 \text{ CoA} + \text{ATP} + 8 \text{ H}_2\text{O} \rightarrow 9 \text{ acetyl-CoA} + 32 \text{ H} + \text{AMP} + \text{PP}$.

The importance of acetyl-CoA in metabolism lies in its ability to undergo a number of different reactions. Some of these are listed in Table 41. Acetyl-CoA is a building block for a large variety of compounds (Table 42). The synthesis of the steroid hormones and of acetylcholine and the synthesis of citric acid are especially important. Citric acid synthesis is the start of the terminal oxidative degradation of foodstuffs in the citric acid cycle. Acetyl-CoA supplied by the stores of carbohydrate and fat enters the cycle by condensation with oxalacetate and is oxidized in stepwise fashion. Acetyl-CoA is thus the substrate of the cycle. Catabolism of the metabolites of the cycle accounts for some two-thirds of total cellular metabolism and energy-supplying processes, as calculated for liver and kidney [218].

Among the usual acyl derivatives of CoA, succinyl-CoA is especially important. This is formed by oxidative decarboxylation of α -ketoglutarate, analogous to the formation of acetyl-CoA from pyruvate. Succinyl-CoA is a mother substance for porphyrin synthesis. It combines with glycine to form α -amino- β -keto-adipic acid, and decarboxylation then produces δ -amino-levulinic acid which is then converted via porphobilinogen into uroporphyrin, coproporphyrin, protoporphyrin, and heme.

The direction of the reaction of the CoA derivatives within the body depends on chemical and physiological equilibria as well as on the organization and function of the individual tissues. Tissues with high energy requirements need large amounts of substrate for the citric acid cycle. These tissues contain large amounts of the condensing enzyme which catalyzes the synthesis of citrate from oxalacetate and acetyl-CoA (Table 43). In addition, the origin of acetyl-CoA is determined by the type of enzyme in the tissues. The liver forms acetyl-CoA from pyruvate, and only to a slight degree from acetoacetic acid-CoA, which it preferably hydrolyzes to free acetoacetate and CoA. The free acetoacetate circulates in the blood and is taken up by other tissues. The kidneys and the heart muscle contain enough enzyme to convert acetoacetate

Table 41. The Reactions of Coenzyme A. From [217]

System	Found in:
<i>Donor Systems</i>	
A. Systems which produce acetyl-CoA	
acetate + ATP + CoA \rightleftharpoons acetyl-CoA + AMP + PP	pigeon liver
oxidation of pyruvate:	
pyruvate + CoA + DPN \rightarrow acetyl-CoA + CO ₂ + DPNH ₂	heart and muscle
(also requires thiamin and α -lipoic acid)	
dismutation of pyruvate:	
2 pyruvate + CoA \rightleftharpoons acetyl-CoA + CO ₂ + lactate	heart and muscle
splitting of acetoacetate	pigeon liver, pig heart, sheep liver
reversal of citrate synthesis	pigeon liver
B. Systems which produce acyl-CoA	
oxidation of α -ketoglutarate (also requires thiamin and α -lipoic acid):	
α -ketoglutarate + DPN + CoA \rightleftharpoons succinyl-CoA + CO ₂	
+ DPNH ₂	cardiac muscle
transacylase (acyl-CoA transferase)	cardiac muscle, kidneys
transfer of benzoyl (synthesis of hippuric acid)	rat liver
transfer of stearyl (synthesis of fat)	rat liver
<i>Acceptor Systems</i>	
A. Acetokinases	
aryl-amino-aceto-kinases	pigeon liver
choline-acetylase	rat brain
histamine-acetylation	pigeon liver
glucosamine-acetylation	pigeon liver
B. Condensations	
Synthesis of acetoacetate	pigeon liver, heart, yeast
Synthesis of citrate	pigeon liver, heart, yeast
Synthesis of pyruvate	
<i>Complex Reactions</i>	
Synthesis and Oxidation of Fatty Acids	heart, liver
Synthesis of steroids and of fats	yeast, liver slices
Synthesis of heme	erythrocytes
Synthesis of isoprene (?)	bacteria, liver

Table 42. Physiological Functions of the End Products of the Metabolism of Acetyl-CoA

End Product	Function
Cholesterol	Parent substance of the sterols and steroids
Steroids	Adrenal cortical hormones, sex hormones
Fat	Storage of energy
Phosphatidic acids	Structural components (e. g., cell membranes)
Hemins	Iron catalysts (biological oxidation, oxygen transport)
Acetylcholine	Components of nerves and cellular membranes
Amino acids	Building blocks of proteins
Acetyl-sulfonamide	Detoxification
Hippuric acid	Detoxification
Citric acid	Intermediate metabolism (oxidation)

Table 43. Condensing Enzyme in Various Tissues of the Rabbit. From Ochoa [175]

Tissues	Enzyme units/mg protein
Liver	0.03
Kidneys	0.2
Brain	0.26
Heart	2.2
Skeletal muscle	0.14

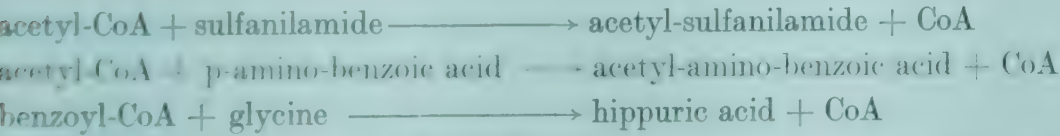
into the CoA derivative, to break the derivative down into two molecules of acetyl-CoA, and to cause condensation with oxalacetate to form citrate. As is well known, both tissues, especially the heart, derive a large portion of their energy from the oxidation of fatty acids.

Pathology of Pantothenic Acid Deficiency

From 1931 to 1933, pantothenic acid was discovered and isolated from sheep liver, and its biological properties were characterized in detail. In the following ten years, its chemical composition was determined and the substance was synthesized [212]. The basic importance of pantothenic acid to life is evident from its widespread distribution in nature and its many biological properties. It is a growth factor for yeast, lactic acid bacteria, and many other microorganisms. It is the dermatitis factor of chickens and the liver-filtrate factor of rats.

Pantothenic acid deficient diets lead to deep-seated abnormalities in all body tissues. The chief abnormality differs from animal to animal. In the rat, the chief findings are dermatitis, inflammation of the mouth and nose, and graying of the fur of dark rats. The heart, kidneys, and adrenals show the chief histologic changes. Paralleling the clinical signs in rats and birds there is a reduction of the bound pantothenic acid in the tissues, especially in the adrenal cortex, the liver, and the heart. The activity of the functions which depend on CoA is correspondingly reduced. Thus, liver slices of pantothenic acid deficient rats show a pronounced reduction of catabolism of pyruvate and of incorporation of acetate into fat and cholesterol. The oxidation of caproic and butyric acids in liver homogenates is inhibited.

The CoA function can be studied in the intact animal by means of special loading tests. Acetyl-CoA reacts with sulfanilamide or p-amino-benzoic acid to form the corresponding acetylated compounds. Benzoic acid is converted by ATP to benzoyl-CoA and reacts with glycine to form hippuric acid:



All these reactions require CoA and their turnover depends on the cellular content of CoA. In deficiency of pantothenic acid, the CoA content of the cells and the turnover of all reactions depending on CoA is reduced. When the normal individual ingests a measured amount of benzoic acid, p-amino-benzoic acid, or sulfanilamide, a large percentage of the ingested compound appears in the urine as hippuric acid, acetylated sulfanilamide, or acetylated p-aminobenzoic acid, respectively. The excretion of these substances is reduced in the presence of pantothenic acid deficiency [220]. The pantothenic acid deficient rat excretes 37% of an injected dose of 2.5 mg p-aminobenzoic

acid in the urine in acetylated form (normal 70%). The same ratio occurs for the other compounds. The ratio of the amount excreted to the amount administered gives an indirect measure of the concentration of CoA in the tissues and therefore of the pantothenic acid content of the body.

Studies of this type have been performed in patients with so-called "burning feet syndrome," which has been described especially in India [221, 222, 223]. Such patients were found to have diminished ability to acetylate p-amino-benzoic acid, the normal (91%) being reduced (to 78%) [224]. Administration of pantothenic acid raised the excretory product to 88% of the ingested substance, with simultaneous improvement of the symptoms, so that this syndrome is considered a manifestation of pantothenic acid deficiency.

The changes in the adrenal cortex are of especial interest in the pathogenesis of the manifestations of pantothenic acid deficiency. Since acetyl-CoA is a building block of the steroids, a deficiency of CoA is followed by diminished synthesis of the steroid hormones. In addition to marked histologic changes in the rat, the adrenal cortex shows reduced cholesterol and corticoid content, and histochemically the ketosteroid reactions disappear [225]. Stress and ACTH in the presence of slight pantothenic acid deficiency favor the appearance of these histologic and biochemical changes in the adrenal cortex.

The disturbances of the nervous system are related to the disturbed ability to acetylate and the resulting diminution of acetylcholine synthesis. However, it is probable that, in addition to disturbed synthesis of acetylcholine, there are also other abnormalities, notably disturbances of all the energy-supplying functions (catabolism of pyruvate, citric acid cycle, etc.). Axelrod et al [226] showed that rats on a pantothenic acid deficient diet showed markedly impaired ability to produce antibodies against human erythrocytes. Pantothenic acid may thus be concerned in the synthesis of proteins.

Clinical Problems

Human deficiency of pantothenic acid has not been described as a clinical entity. Probably, the human organism is especially well protected against the development of such deficiency. The intestinal bacteria produce optimal amounts of pantothenic acid, and thus protect against disturbances of metabolic processes dependent on pantothenic acid. Occult deficiency may occur, however, as the result of other metabolic disturbances: malnutrition, protein deficiency, deficiency of other B vitamins, altered intestinal flora following prolonged antibiotic therapy, increased vitamin requirement during disease, chronic alcoholism, pregnancy, growth. The special "burning feet syndrome" has been described as a deficiency of pantothenic acid: this seems to be identical with erythromelalgia tropica, nutritional melalgia, and the paresthetic-causalgic syndrome [222, 223]. The chief complaints are severe burning of the soles of the feet and lightning pains in the shins. The legs may show erythema, cyanosis, and desquamation. The pains are especially pronounced at night in the warmth of the bed. They are often thought to be intermittent claudication. Successful therapy with pantothenic acid confirms the deficiency of pantothenic acid. The deficiency may be demonstrated by the lack of ability to convert p-amino-benzoic acid to its acetylated product (see p. 143). Similar types of pain have also been described in Europe and been shown to respond to therapy with pantothenic acid [8]. Leg cramps of pregnant women are also said to respond to pantothenic acid therapy. Kühnau [8] suggests a

possible relationship between night burning of the feet in elderly people and the Swift-Foot disease of children, and deficiency of pantothenic acid.

If pantothenic acid deficiency is suspected, multivitamin therapy with added pantothenic acid is indicated. The daily requirement of pantothenic acid is given as 2 to 4 mg [227]. More is required during pregnancy and in the first months of life. Vitamin preparations containing adequate amounts of pantothenic acid are available. Green vegetables, liver, and the yellow of egg can also be employed in therapy because of their high pantothenic acid content. Cereals contain little pantothenic acid.

Intermediate Metabolism and the Thyroid Gland

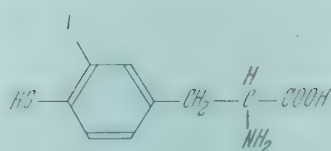
In 1821, soon after the discovery of iodine by Gay-Lussac, the Swiss physician Coindet used iodine in the treatment of goiter [228]. Coindet had found that the active principle of the algae used for centuries for treatment of goiter was free iodine. In the middle of the 19th century, it was discovered that extirpation of the thyroid gland was followed by experimental myxedema, and that an extract of the thyroid gland remedied this condition. The relationship between iodine and thyroid function was first shown in 1895 by Baumann's discovery of large amounts of organically bound iodine within the thyroid gland. Baumann suggested that the endocrine function of the thyroid was related to the iodine content of the gland. In 1901 Oswald isolated an iodine-containing protein, "thyroglobulin", from the thyroid [230]. In 1916, Kendall isolated an iodine-containing amino acid, thyroxin, from thyroid extract [231]. Harington described the structure and synthesis of thyroxin in 1926 [232]. In recent years, Gross and Pitt-Rivers [223, 240 a] isolated 3', 3,5-triiodothyronine and other derivatives of thyronine.

Thyroglobulin, thyroxin, or triiodothyronine can replace thyroid function in a thyroidectomized individual. The active components of thyroglobulin are the two peptide-bound amino acids, thyroxin and triiodothyronine which are hydrolyzed to their free forms within the body. Thyroglobulin is not a hormone but merely the carrier of an active group. The hormones of the thyroid gland are free triiodothyronine and thyroxin. These hormones are synthesized within the thyroid and secreted by it into the body.

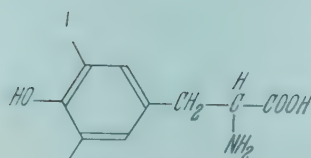
The Circulation of Iodine

The animal body contains iodine in various forms, including inorganic iodine as iodide and elementary iodine (in traces), a number of low-molecular organic iodine-containing compounds, and an iodine-containing protein (thyroglobulin).

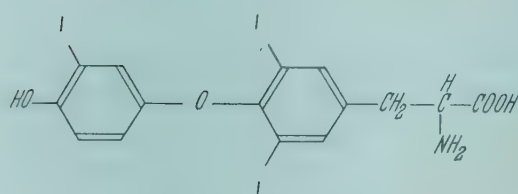
The low-molecular iodine-containing compounds are involved in the intermediate synthesis and degradation of the thyroid hormones. Chemically, they are present partly free, partly as protein-bound amino acid derivatives or the transamination product of L-tyrosine, and mono-iodohistidine. The simplest derivative of L-tyrosine is 3-monoiodotyrosine. This is present exclusively in the thyroid gland and is chiefly present in bound form as a building block of thyroglobulin. It occurs in free form only in trace amounts. Monoiodotyrosine, which forms about 0.1 to 0.2% of the total globulin, contains 10 to 15% of the total iodine of the thyroid in rats and is an intermediate product in the synthesis of thyroid hormone. Another substance, 3,5-diiodo-



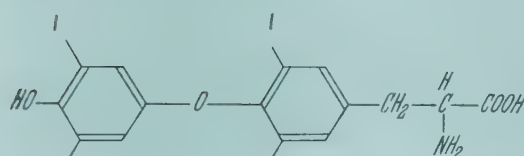
Monoiodotyrosine



Diiodotyrosine



Triiodothyronine

Tetraiodothyronine
(Thyroxine)

tyrosine, is present almost entirely in bound form in thyroglobulin, forming 0.54% of the latter and containing 30% of the total iodine of thyroglobulin. This substance was discovered by Drechsel [234] in *Gorgonia verrucosa* Pallas and therefore was originally called "iodo-gorgoic acid." Like monoiodotyrosine, it is an intermediate product in the synthesis of the thyroid hormone. This substance is of importance in the treatment of hyperthyroidism, for it is deiodated by animal tissues and its free iodide becomes pharmacologically active. Two iodine-containing transamination products of diiodotyrosine, which are probably degradation products, are 3,5-diiodo-phenyl-pyruvate and 3,5-diiodo-phenyl-lactate [7].

The condensation of two molecules of tyrosine leads to thyronine, from which the two main thyroid hormones are formally derived (3', 3,5-triiodothyronine and thyroxine). Thyroxine, which is 3', 5', 3,5-tetraiodothyronine, is insoluble in acids and soluble in alkaline solutions. In contrast to diiodotyrosine, thyroxine can be extracted from alkaline solution by means of water-saturated butanol. Thyroxine can be isolated from biological media (plasma, alkaline protein hydrolysate) either as an acid-insoluble fraction ("acid-insoluble iodine") or as a butanol extract. For quantitative

determination, the fraction is ashed and free iodine is determined in a colorimeter (p. 154). The chemical properties of 3', 3,5-triiodothyronine resemble those of thyroxine. Both compounds can be separated by means of paper chromatography from each other and from other iodine-containing amino acids. In addition to these compounds the following thyronine derivatives have been identified in the thyroid as part of thyroglobulin and in the plasma of the rat: L-3,3'-diiodothyronine and 3,3',5'-triiodothyronine. Furthermore, triiodothyroacetic acid has been found in the kidney and triiodothyropyruvate and tetraiodothyropyruvate in the bile [239, 240 a].

3', 3,5-triiodothyronine and thyroxine are present within the thyroid gland both in free form and in peptide linkage. The bound form of thyroxine is present as part of the thyroglobulin (0.21%) and binds some 10% of the total iodine of the protein. Table 44 gives the proportion of 3', 3,5-triiodothyronine in the thyroid gland. The concentration of the free hormones in the gland is not known.

The organic iodine of the plasma is composed of thyroxine and 3', 3,5-triiodothyronine, with other iodinated substances in minute amounts [7 a, 237, 238, 239]. Thyroxine is present in the serum in loosely bound form, from which it can be extracted by butanol but not by dialysis. The substance which carries the hormone is a lipoprotein which moves with the α -globulins ("inter-alpha-region") on electropho-

Table 44. Average distribution of the various iodine compounds in the normal human thyroid. In untreated hyperthyroidism, the total iodine content is reduced, but the distribution is the same. Modified from [236]

Compound	% of total iodine content
Thyroxin	35
Triiodothyronine	8
Diiodotyrosine	25
Monoiodotyrosine	17

rests at pH 8.5 [240]. A small portion of the total thyroxin is present in free form in the blood. 3', 3,5-triiodothyronine is also present in the plasma in the α_1 , or "inter-alpha" region [241, 242]. 3', 3,5-triiodothyronine and thyroxin constitute approximately 6 to 8 % of iodine per 100 ml plasma, calculated as organic iodine. Approximately 10-20% of plasma iodine is iodide, 70% is thyroxin, and the remainder is 3', 3,5-triiodothyronine and other iodinated substances [240 a]. Erythrocytes do not contain organic iodine.

The concentrations of the individual compounds in the tissues is not known. From the organic iodine content of the tissues, the concentration of total hormone is calculated to be approximately 10^{-7} to 10^{-8} mols/liter [243].

In addition to the iodine-containing tyrosine derivatives, the thyroid gland of the dog and the rat contains 2- or 4-monoiodohistidine. These are present only in bound form as part of the thyroglobulin and contain 1 to 3% of the iodine of thyroglobulin [244]. The monoiodohistidines have no known function.

Of the natural iodine-containing proteins, only thyroglobulin is of importance in human metabolism [244]. This substance is found only in the colloid follicles of the thyroid gland. It cannot be demonstrated normally in the plasma by either immunologic or chemical methods. Roche and his coworkers isolated a pure thyroglobulin from the thyroid glands of hogs, and showed that it was a homogenous substance both on ultracentrifugation and on electrophoresis [244]. Fractional precipitation with neutral salts separated three components, which were thought to be different physical forms of the compound. Human thyroglobulin behaves in the same way. According to its solubility thyroglobulin belongs to the globulins with a molecular weight of 680,000. Thyroglobulin is not pure protein, but a glycoprotein [240 b]. Its chief chemical property is its ability to bind large amounts of iodine, which it does by way of tyrosine, tyrosine derivatives, and histidine (Table 45). Under normal con-

Table 45. Iodine-containing amino acids and their parent substances in hog thyroglobulin. Modified from [244]

Amino acid	% of protein	% of total iodine of protein**
Tyrosine	3.12	—
Monoiodotyrosine	0.1-0.2	10-15
Diiodotyrosine*	0.54	} 90-95
Thyroxin*	0.21	
Triiodothyronine	?	?
Histidine	2.23	—
Monoiodohistidine	—	1-3

* Content varies from animal to animal.

** Total iodine content varies; the average is approx. 0.48%.

ditions, the concentration of the individual amino acids in the thyroid gland is relatively constant. There is little variation in the amount of tyrosine in pigs, oxen, dogs, and other animals. Patients with goiter show changes in the amino acid concentrations, especially in the tyrosine and cysteine values. The thyroglobulin obtained from a human colloid goiter shows more tyrosine than that in hyperplastic, hyperactive glands. However, the concentration of the other amino acids is constant. The amount of iodine can vary from 0.87% to 0.18%. The ratio of thyroxine iodine to total iodine is constant among different species (0.24 to 0.32) and is independent of the total iodine content of the protein.

The biosynthesis of triiodothyronine and thyroxine takes place on pre-existing thyroglobulin [245] and demands the prior synthesis of globulin. The synthesis of the protein and of the hormone can be considered as separate processes which follow each other. The synthesis and degradation of the globulin follow the general rules of protein metabolism. The synthesis of the hormone takes place on the tyrosine units of the specific globulin produced by the thyroid cells. The synthesis takes place by means of stepwise iodination followed by condensation of two molecules of diiodotyrosine to thyroxine or of monoiodotyrosine with diiodotyrosine to triiodothyronine, respectively. The presence of reactive inorganic iodine is necessary for iodination to occur.

The uptake of inorganic iodine from the circulation is accomplished by a concentrating mechanism specific for thyroid tissue. Other endodermal tissues (stomach, tissues derived from the branchial clefts, salivary glands) also possess this mechanism to a small degree. Radioactive iodine (I^{131}) administered to the mother can be found in the fetal thyroid from the 12th week of life on. The thyroid of the fetus develops its typical histological structure from the 16th week on.

The extraordinary avidity of the thyroid for iodine is shown by the tremendous proportion of total body iodine contained within the thyroid. The thyroid contains 12 to 15% of the total iodine of the body (some 40 mg iodine per 100 gram fresh weight of gland), although the weight of the thyroid is only 0.03% of the total weight of the body. The concentration of iodide in the cells of the thyroid is thus 100 to 300 times that in all other body cells. Iodide itself comprises only 2% of the total iodine content of the gland.

The mechanism of uptake and fixation of iodide is not known. In cases of thyroid neoplasm, as the tissue becomes less and less differentiated, the ability to take up iodide is lost.

Fixation of iodine is influenced by the capacity of the thyroid cells, the plasma concentration of iodide (p. 151), and the action of the thyrotropic hormone of the anterior pituitary. In addition, two types of drugs affect iodine uptake and fixation ("thyrostatic drugs"). The first group includes the ions of thiocyanate, perchlorate, chlorate, periodate, and iodate. These drugs inhibit the concentration of iodide by the thyroid cells and displace iodide already present within the cells - i. e., they "discharge" the inorganic iodine within the thyroid. The mechanism is not simply one of competitive inhibition, and is not really understood. The second group of thyrostatic drugs are the thio-amido compounds, which inhibit fixation of iodide without affecting iodide uptake within the cell.

Iodide which is taken up by the gland is first oxidized to elementary iodine before it is fixed (Fig. 45). The oxidizing enzyme is probably a hydroperoxidase. Stanbury and Hedge [246] found that sporadic cretins were able to concentrate iodide in the gland but were unable to convert iodide into iodine. In such patients, apparently, the oxidizing enzyme is lacking or deficient. Elementary iodine is attached directly.

without the intervention of a specific enzyme, to the structurally preferred tyrosine residues of thyroglobulin. Monoiodotyrosine and then diiodotyrosine are thus formed. Fifteen minutes after the injection of radioiodide into a rat, almost 95% of the iodide is bound to the protein of the gland, with 82.7% in the diiodo fraction and 12.5% in thyroxine. After 50 hours, the activity is differently distributed, with 71% in diiodotyrosine and 29% in thyroxine [235]. Chaikoff and Taurog [247], in a similar experiment, showed that ten minutes after the injection the specific activity of the monoiodotyrosine is at its peak, and then falls below the activity of the diiodotyrosine after 24 to 48 hours. Labelled tyrosine appears in the plasma only after the radioactivity has been transferred through the compounds within the thyroid gland [248].

Two molecules of diiodotyrosine condense to liberate a molecule of alanine and form thyroxine. This condensation is probably effected by means of a hydroperoxidase. In vitro with hydroperoxidase and hydrogen peroxide the condensation can be carried out with a good yield. De Robertis and Grasso [249] showed that the thyroid contains a hydroperoxidase. The synthesis of thyroxine is shown schematically in Fig. 45.

The final step of the synthesis of thyroxine, the condensation of diiodotyrosine as N-acetyl-diiodo-tyrosyl-glutamate to the corresponding tyrosine derivative, has been accomplished in vitro under physiological conditions [250]. Elementary iodine accelerates this reaction. In the presence of elementary iodine, the entire thyroxine synthesis can occur

without cellular elements in vitro. In addition, the injection of elemental iodine into thyroidectomized animals has a thyroxine-like effect. Despite these facts, the synthesis in vivo differs fundamentally from that in vitro by its much greater velocity and by the fact that following hypophysectomy synthesis ceases at the stage of diiodotyrosine. Thyrotropic hormone thus accelerates the synthesis.

In contrast to these facts concerning the synthesis of thyroxine, little is known concerning the bio-synthesis of 3', 3,5-triiodothyronine [245]. However, triiodothyronine

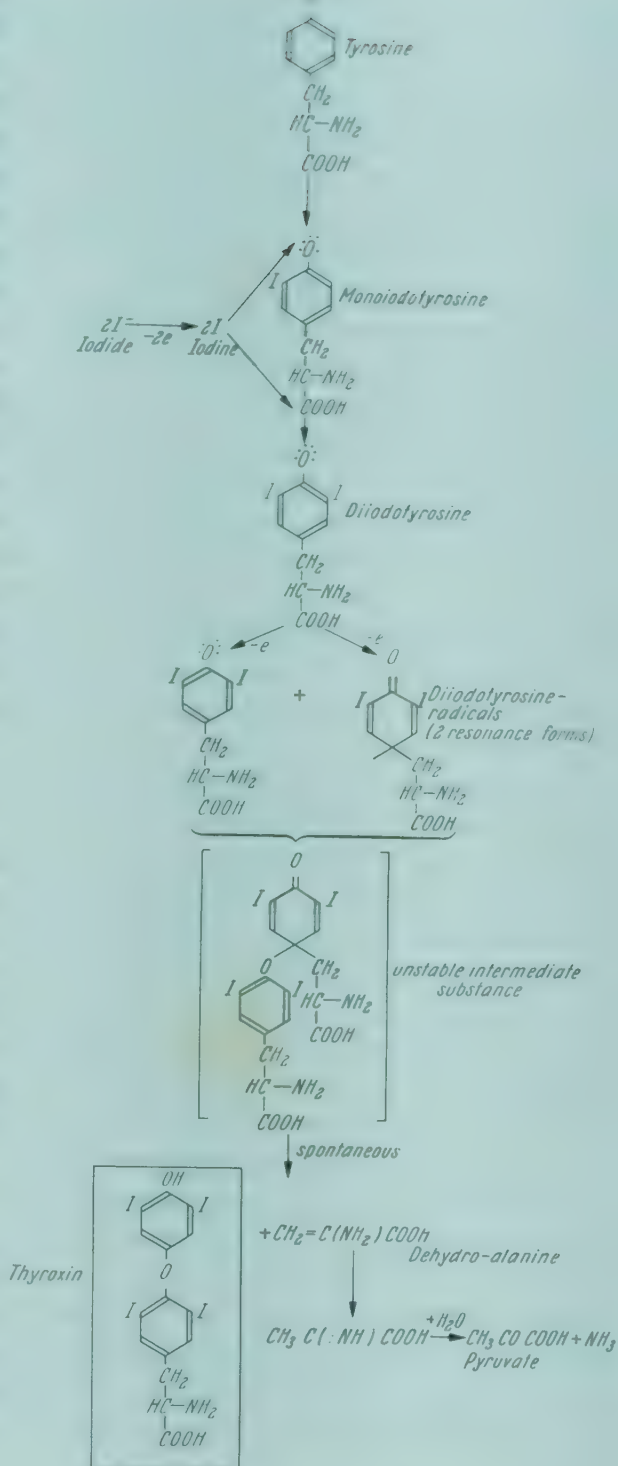


Fig. 45. The Synthesis of Thyroxine.

is formed at the same time and just as rapidly as thyroxin. 3,3'-diiodothyronine and 3,3,5-triiodothyronine are probably synthesized by condensation of monoiodotyrosine and diiodotyrosine in appropriate proportions [7 a].

Iodinated thyroglobulin is broken down within the thyroid follicles to lower peptides and amino acids by means of a proteolytic enzyme system. This proteolytic activity is increased by thyrotropic hormone and inhibited by iodide. As a result of the proteolysis, diiodotyrosine, thyroxin (each comprising 0.5% of the total iodine content of the rat), monoiodotyrosine, and triiodothyronine are present in the thyroid as free amino acids [251]. Free diiodotyrosine and monoiodotyrosine are further degraded by deiodinating enzymes to tyrosine and free iodide, and are available again for the synthesis of the hormones. Therefore, neither substance occurs in the serum. Free thyroxin and triiodothyronine, on the other hand, pass through the thyroid cells into the blood stream by diffusion, with a concentration gradient of 100:1 for thyroxin.

The chief site of hormone synthesis is the thyroid gland. Morton has shown, however, that small amounts of thyroxin can be formed outside of the thyroid [252]. Whether this is also true for triiodothyronine is not known. The process of synthesis in other tissues is the same in principal as that in the thyroid. However, the amount synthesized outside the thyroid is too small to supply, for example, the thyroid requirement of a patient with myxedema. Bansi [1] has suggested that increased extra-thyroidal synthesis may account for the failure of thyroidectomy in certain cases of hyperthyroidism.

Degradation of diiodotyrosine and monoiodotyrosine by deiodination occurs not only in the thyroid gland but also in the serum, the liver, and the kidneys. The rate of deiodination in these tissues is small. In addition diiodotyrosine undergoes transamination to form 3,5-diiodo-p-hydroxy-phenyl-pyruvate and 3,5-diiodo-p-hydroxy-phenyl-lactate. The further fate of these substances is not known [251]. The tyrosine and inorganic iodine set free during deiodination pass into the general iodide and amino acid circulation. Thyroxin and, to a smaller extent, triiodothyronine, are excreted in the bile as their glucuronic acid conjugates [253, 254]. In the coupling of glucuronic acid and thyroxin, uridine diphosphate glucuronic acid serves as the donor of glucuronic acid [255]. Dehalogenation and deamination plus oxidative decarboxylation are other pathways of degradation [7 a].

The body gets its iodine chiefly from the diet, to a lesser extent by inhalation from the air or absorption through the skin. The optimal amount of iodine for man is given as 150 γ per day [256]. Deficiency or excess of iodine lead to well-known disturbances of thyroid function. Dietary iodine is present chiefly as inorganic iodide and is rapidly and completely absorbed in this form. Little iodide appears in the stool. The rate of absorption in the intestine is normally about 5% of the dose per minute [257], but is directly proportional to the level of thyroid activity (less absorption in myxedema).

The thyroid gland takes up inorganic iodine, incorporates it into the thyroid hormones, and secretes these into the blood stream. The amount of hormone secreted daily is about 110 γ of thyroxin (= 70 γ thyroxin iodine) [256]. There are no data for triiodothyronine, but the order of magnitude seems to be the same as for thyroxin [258]. Either hormone can substitute for the other. The excretion of the hormones in the urine and bile can normally be ignored. The hormones pass via the circulation to their site of action at the body cells. The non-protein-bound hormone iodine of the blood, which forms only a small portion of the total hormone iodine, is in equilibrium with the tissue hormone iodine by means of diffusion [259].

In the periphery, protein-bound iodine is again broken down to inorganic iodine after it has performed its function. This then passes into the blood stream and is either excreted in the urine or again taken up by the thyroid gland.

The distribution of absorbed iodine is independent of the route of introduction of the iodine into the body and depends on the size of the dose. A physiological dose of iodine (0.01 to 25 μ iodine) given orally is absorbed, mixes with the blood iodide, and follows as a tracer the normal iodine circulation without affecting the normal iodine concentration of the blood (less than 1 μ o). Pharmacological doses (500 μ to 1 mg iodine) cause appreciable elevation of the blood iodide concentration and have pharmacological effects. Iodine is distributed along a concentration gradient through the blood stream and into the extracellular spaces. The iodide ion behaves like the chloride ion, passing into all tissue spaces and transudates. There is free exchange in both directions between blood and tissue lymph [7 a]. If the blood iodide is markedly elevated by pharmacological doses of iodine, all the organs of excretion (kidneys, liver, stomach, lungs) and the thyroid glands increase their activity above normal until the normal concentration of iodide in the blood is restored.

The amount of iodine fixation within the thyroid is determined, among other factors, by the plasma iodide level. Fifty percent of a physiological dose of iodine enters the thyroid gland of man 48 hours after the dose. On the other hand, only 0.5% of pharmacological doses are found in the gland after 24 hours. In the latter case, the thyroid is inundated with iodide as a result of the high blood iodide and is fully saturated 10 minutes after the oral dose. No more iodide is taken up. The result of the saturation with iodide is an inhibition of the mechanism which fixes iodide and of the synthesis and secretion of thyroxin. The normal thyroids of dogs, rats, and rabbits, for example, cannot take up more than 10 to 20 mg of iodine per 100 gram fresh weight [257]. In the rat, saturation of the gland and resulting inhibition of fixation and synthesis occur when the blood iodide concentration is 20 to 35 μ o (Table 46). Wolff and Chaikoff [260] used the term "homeostatic auto-regulation of hormone synthesis." This degree of saturation is not reached with physiological doses of iodine, although repeated administration of moderate amounts causes some reduction of the capacity of the thyroid to take up iodide, and partial inhibition occurs. Table 46 lists the relationship between blood iodide level and fixation of iodide by the thyroid.

The elevated blood iodide following pharmacological amounts of iodine is compensated for by increased excretion. The amount excreted is proportional to the size of the dose ingested. The excretion curve is exponential.

The clearance of iodide by the normal kidney is virtually constant over a plasma iodide range from 10 mg% to 0.02 μ o (normal is 1 μ o) [261]. The clearance averages 34 cc of plasma per minute, with a range from 11 to 55 cc. This value is approximately one-fourth of the glomerular filtration rate, so that it may be assumed that approxi-

Table 46. Influence of the blood level of iodine on the fixation of iodine in the thyroid gland. From Leblond [257]. Determinations made at the time of maximal thyroid uptake of I^{131}

Amount of I^{131} injected mg/kg body weight	Blood level of I^{131} mg/100 cc	I^{131} content of thyroid gland mg/100 grams	Ratio of thyroid iodine to blood iodine
50	7.31	23	3
5	1.25	16	13
0.1	0.032	7	219
0.005	0.003	1.6	533

mately three fourths of the iodide in glomerular urine is reabsorbed by the tubules [256]. The kidney is unable to retain iodide even when the plasma iodide is low, a fact which is unfavorable in cases of deficient iodine intake. The high thyroid avidity for iodine, however, compensates for these losses. Since the excretory function of the kidneys is not affected by disturbed iodine metabolism, the amount excreted depends on the degree of activity of the thyroid gland itself. The more iodide (I^{131}) the thyroid retains, the less appears in the urine. The relationships between the iodide uptake in the thyroid and the urinary excretion of iodide are important in clinical diagnosis.

The amount of iodide excreted in the lungs is in direct proportion to the level of blood iodide. The measurement of I^{131} in the exhaled air following a tracer dose of I^{131} can be used as a test of thyroid function [257]. The stomach takes up pharmacological doses of radioiodide to a degree which corresponds to its activity. After the injection of histamine, up to 30% of an injected dose of iodine is secreted in the stomach within 30 minutes. The iodide ion behaves like the chloride ion, so that hydriodic acid appears in the gastric juice. This iodide is again absorbed in the intestinal tract. The secretion and subsequent reabsorption of iodide is considered to constitute an intestinal circulation of iodide, with the function of storage. The iodide excreted in the bile also enters this circulation [257].

Even when the body is flooded with high doses of iodide, the uptake of iodide by the various organs is negligible as compared with the amount concentrated in the thyroid, and the blood iodine level returns to normal within 24 hours. No special concentration of iodide occurs in the hypophysis, ovaries, or adrenals, as measured with labelling techniques.

The balance of iodine is determined by the dietary iodine, the utilization of iodine as thyroid hormone in peripheral metabolism, and the excretion of iodide (Fig. 50, Table 49). Variations in the intake of iodine in the diet are largely compensated for by storage in the thyroid glands which guarantees constant synthesis and secretion of thyroid hormone. Anatomically, there may be increase, as in simple goiter, or decrease of the functional surface area of the glands according to the daily intake of iodine. The utilization of the hormone, and therefore its synthesis and secretion in the thyroid glands, depends on the peripheral metabolic rate [256]. A simple mechanism governs this relationship: Reduction of the level of thyroid hormone in the blood stimulates the anterior pituitary to increased secretion of the thyrotropic hormone, which increases the activity of the thyroid, so that the synthesis and secretion of hormone are increased. It is possible to show, both *in vivo* and *in vitro*, the acceleration of iodide uptake and fixation, the condensation reaction, and the secretion of hormone. Under the action of large amounts of thyrotropic hormone, the thyroid shows hypertrophy, hyperplasia, and vascularization as signs of increased activity. An increase of the thyroid hormone in the blood causes reduced secretion of the thyrotropic hormone of the anterior pituitary, with subsequent reduction of the activity of the thyroid.

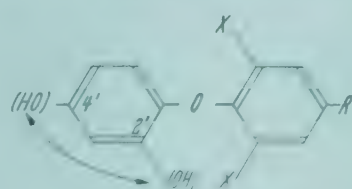
Physiological Action of the Thyroid Hormone

Since the experiments of Magnus-Levy in 1895 [51], it has been known that hyperfunction of the thyroid is associated with an increased uptake of oxygen, and hypofunction with decreased uptake of oxygen. These phenomena suggested a basic effect of thyroid hormone on bodily function and through the years have been fundamental

in the study of thyroid function. Other actions of the hormone on various biological substrates were explained on this basis. Better understanding of the relationship between thyroid hormone and cellular respiration has come in the last few years (p. 95).

The problem of mechanism of action is complicated by the fact that the hormone is present in the blood in two main forms, thyroxine and 3,3,5-triiodothyronine. Both substances are qualitatively the same, but 3,3,5-triiodothyronine has a stronger physiological action than thyroxine [262]. Furthermore, the question remains whether both substances are ultimately converted in the tissues into an active form. Since 3,3,5-triiodothyroacetic acid has been found to be the most active compound, it is considered to be the active form of the hormone [7 a].

There is no specific chemical structure which underlies the biological activity of the thyroid hormones. The maximal action of the hormonal structure is associated with an iodine substitution at 3', 3,5 position of the nucleus no matter what side chain is present [7 a]. Furthermore, chemical changes of the hormone molecule produce compounds which have the same physiological action as thyroid hormone, although their activity is quantitatively weaker [263, 264]. Thus, if the iodine atom is replaced by bromine, tetrabromothyronine results; this has about half the activity of thyroxine. Tetranitrothyronine has only $1/3000$ the activity of thyroxine. The following is the minimal structure essential for the physiological activity of the hormone:



$X = \text{I, Br, Cl, NO}_2$.

R must contain either COOH or NH_2 .

OH can be in either the 2' or the 4' position.

Minimal structure necessary for thyroxine activity [263].

Further changes in the structure of the hormone leads to substances which differ qualitatively from the thyroid hormones. As is well known, 2,4-dinitrophenol has the same effect on oxygen utilization as thyroxine [265, 266]. Physiologically, however, it does not replace the hormone. Treatment of myxedema with dinitrophenol causes normalization of the basal metabolism and produces creatinuria, but there is no improvement of the other symptoms or signs. Similarly, dinitrophenol cannot replace thyroxine in the growing organism.

Structural antagonists to thyroid hormone were developed by Woolley [267] and MacLagan [268]. These act by competitive inhibition, displacing the hormone from its place of action. One of the strongest antagonists is the *n*-butyl ester of 4-hydroxy-3,5-diiodo-benzoic acid.

The chemical structure of the hormone suggests certain hypotheses concerning its activity. Kendall had already suggested that the hormone affects the redox processes within the cells [269]. Niemann showed that the basic structure of the hormone is characterized by its ability to be converted to a quinoid [270]. However, the site of action in the redox systems is not known. The administration of thyroid extract or thyroid hormone increases the oxygen utilization; thyroidectomy or the use of anti-thyroid drugs reduces the oxygen utilization. These facts have been shown in isolated tissues, in tissue slices, in tissue homogenates, and in the mitochondrial fraction of cells. Human metabolism is subject to the same effects. An increase of oxidative processes is

characterized by increased utilization of oxygen and by increased utilization of substrates, which are synthesized from carbohydrates, fats, or amino acids. The high consumption of foodstuffs in hyperthyroidism has long been familiar to the clinician. There is pronounced loss of weight. Signs of increased utilization of oxygen are lacking when insufficient substrate is available. Thus, when there is a deficiency of vitamin B₁, the administration of thyroid hormone does not cause elevated oxygen utilization, for the deficient decarboxylation of α -keto-carboxylic acids produces insufficient substrate for oxidation [271].

In hyperthyroidism, the order of magnitude of the energy-requiring reactions does not correspond to the high oxygen utilization. The energy yield of cellular respiration is reduced.

Recent investigations have studied the quantitative relationships between oxidation and the energy-producing reactions (oxidative phosphorylation). There is a close relationship between the utilization of oxygen and the synthesis of high-energy phosphate bonds: normally, 60 to 70% of the theoretical amount of chemical energy is transformed into high energy phosphate. Martius and Hess [43] showed that thyroid hormone affects oxidative phosphorylation *in vitro* and *in vivo*, and their results were confirmed by others [47 a, 47 b]. Study of oxidative phosphorylation in isolated tissues (liver, diaphragm, kidney) obtained from animals with experimental hyperthyroidism shows that the yield of high-energy phosphate in relation to oxygen utilization is reduced (p. 94). The opposite is true in hypothyroidism. The various results of this effect on oxidative phosphorylation have already been discussed. The effect of pathological levels of thyroid hormone (hyper- or hypothyroidism) on this process which is fundamental in the production of energy can produce a multitude of secondary manifestations. The effects are different in the growing individual from those in the adult. Children develop cretinism or show hyperthyroid tall, thin growth. The adult shows myxedema or thyrotoxicosis. Growth and differentiation are extremely complex events: a number of different factors (hormones, vitamins, amino acids, other building blocks) are involved in normal growth and differentiation. The thyroid hormones cannot be considered to be differentiating hormones *per se*. The disturbances of growth can be directly explained on the assumption that the thyroid regulates energy-supplying processes which are fundamental to growth.

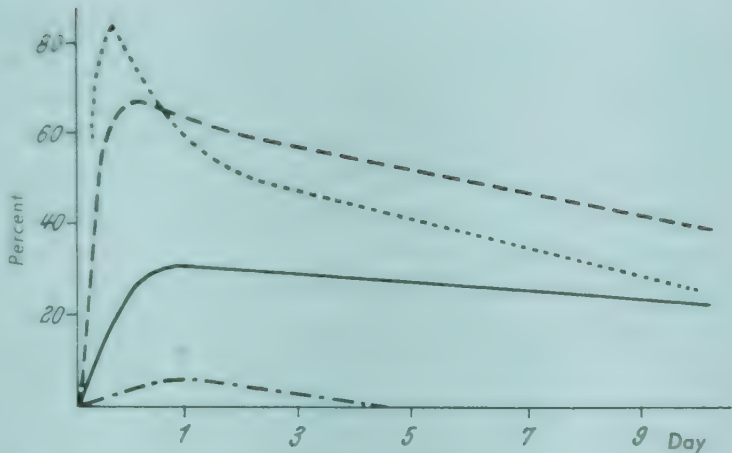
Diseases of the Thyroid Glands

Diagnostic Methods

The diagnosis of disturbances of thyroid function depends on the metabolism of iodine in the body and on the clinical and biochemical effects of the thyroid hormones. Iodine metabolism can be measured by chemical and physical means. The determination of protein-bound plasma iodine and of the total tissue iodine is of especial importance. Veil and Sturm introduced a clinical method for the determination of the blood iodine as long ago as 1925 [272]. Modern methods of determination depend on the catalytic properties of iodine in redox reactions. The methods of Sandell and Kolthoff [273] and Chaney [274] are based on the fact that small amounts of iodine ions catalyze the reduction of yellow Ce^{4+} ions to colorless Ce^{3+} ions by arsenious acid. The method is extremely sensitive [275]. In the normal, the serum protein-bound iodine is 6 to 8 γ %, and the serum inorganic iodine (iodide) is 1 γ %. The daily urine contains approximately 144 γ of iodide [256].

Fig. 46. Uptake of radioactive iodine by the thyroid as a percentage of the administered dose after a single dose. From Horst and Harnack [259].

- severe hyperthyroidism
- moderate hyperthyroidism
- euthyroidism
- hypothyroidism

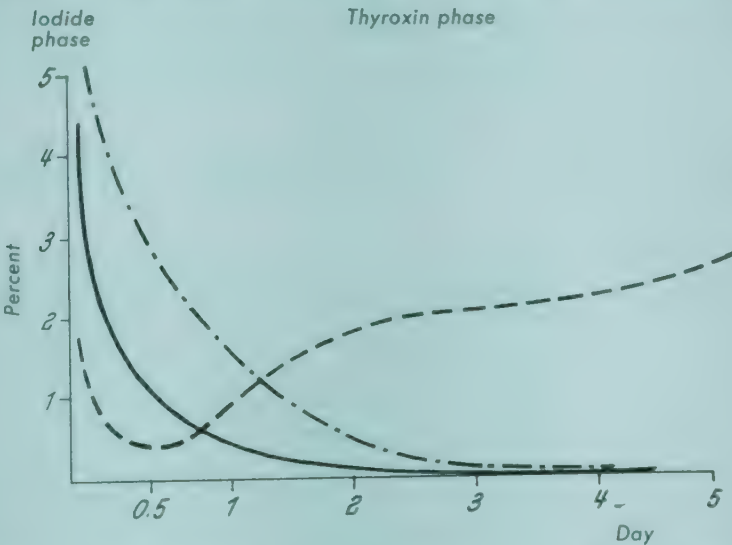


The physical method of measuring iodine metabolism employs I^{131} . The half-life of this isotope is 8.0 days. In six hours, 2.14% of the isotope decays. Following oral or parenteral administration of a tracer dose of I^{131} , the distribution of the isotope is determined either by in vivo counting over the thyroid gland or by determination of the radioactivity in the blood or the urine [1].

The amount of I^{131} administered must be physiological (less than 70 γ iodide), and, of course, there is a limit to the amount of radioactivity which may be given safely. Radioactivity is potentially carcinogenic; in addition, in large amounts, it may cause changes of the permeability of the thyroid cells, which may lead to the secretion of

Fig. 47. The serum iodine after a single dose of I^{131} , graphed as a percentage of the administered radioactive iodine per liter of serum. From Horst and Harnack [259].

- euthyroid
- hypothyroidism
- hyperthyroidism



unphysiological amounts of thyroid hormone and thyroid protein into the blood. The usual tracer dose is given as carrier free I^{131} with 0.01 γ of iodide per 100 mc. The dose used is 30 to 60 μ c of I^{131} .

The function of the thyroid can be studied by various means, including direct measurement of the rate and amount of iodine uptake in the gland, determination of the iodine excretion, and determination of the rate of hormone secretion. The amount and rate of uptake of radioactive iodine by the thyroid is routinely determined in vivo 2, 12, or 24 hours after the tracer dose is given. The curve of iodine uptake with time is depicted in Fig. 46 and Table 48 [259]. The maximal uptake of iodine by the thyroid can also be estimated by the excretion of iodide in the urine after the tracer dose, as the difference between that administered and that excreted [1]. Both methods give the activity of the thyroid in the "iodide phase"; i. e., in the interval

during which the I^{131} is partly taken up by the thyroid and partly excreted in the urine [259]. In contrast, there is the "thyroxin phase" or "hormone phase," which corresponds to the secretion of radioactive organic hormone iodine into the blood (Fig. 47). In all these methods, the status of the thyroid gland is correctly determined by the results only if the other organs are taken into consideration: kidney function (iodide clearance), plasma proteins (abnormal, for example, in nephrosis), and the extracellular space (edema).

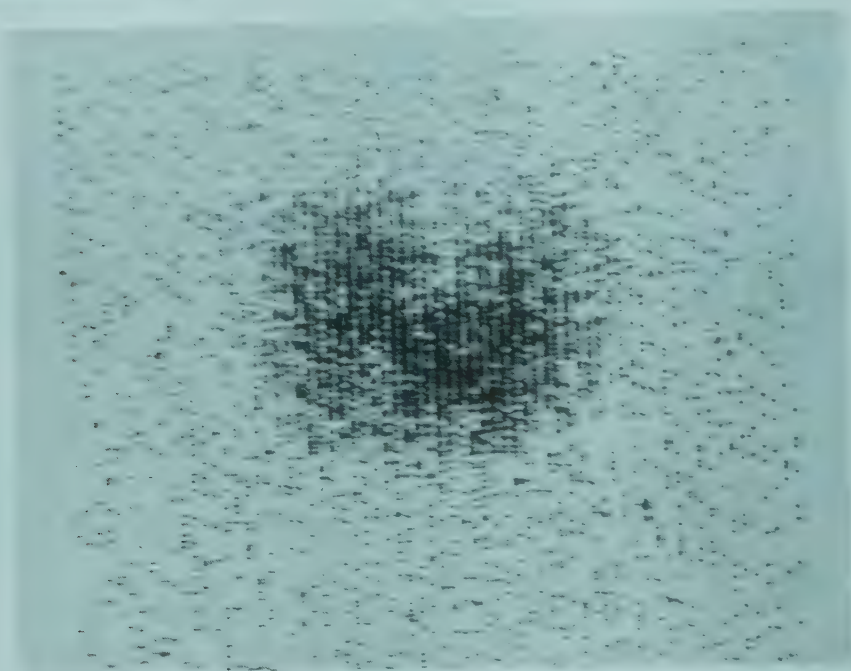


Fig. 48. Scintigram of a normal thyroid ($25 \mu c I^{131}$). (Case of Dr. Scheer, Czerny Krankenhaus, University of Heidelberg.)

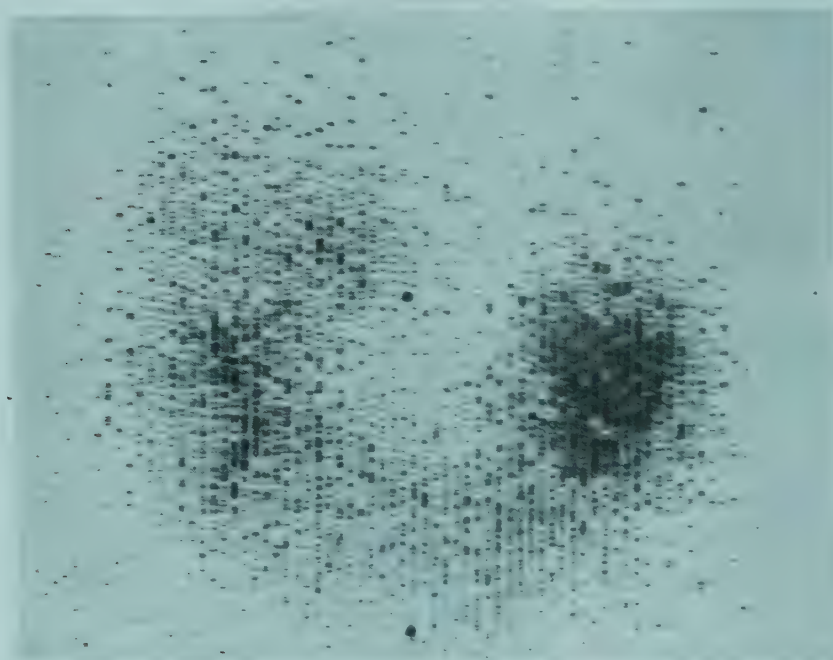


Fig. 49. Scintigram of a markedly enlarged diffuse toxic goiter ($30 \mu c I^{131}$). Pronounced I^{131} concentration in the left lobe, indicating a circumscribed nodule. (Case of Dr. Scheer, Czerny Krankenhaus, University of Heidelberg.)

The structure of the thyroid can be analyzed by localizing hyperactive and hyporeactive areas within the total thyroid gland [259]. *In vivo* counting with appropriate instruments permits such study, and a picture of radioactivity within the gland can be made with scanning devices (Fig. 48 and 49). The amount of radioactivity depends on the level of function of the thyroid. Changes in function may be due to diffuse as well as localized changes of thyroid tissue, so that such scanning methods are of great value in diagnosis.

Clinical diagnosis is also based on the basal metabolic rate, which is a function of the direct effect of thyroid hormone on energy metabolism (p. 152). Additional methods are the use of radioactive iodine, the determination of protein-bound iodine in the serum, the serum cholesterol, and the excretion of creatinine.

Historical Aspects

The classical signs of hyperfunction and hypofunction of the thyroid were known long before the thyroid hormones were discovered. In 1850, Curling in England described atrophy of the thyroid in sporadic cretinism [276]. Nine years later, Schiff in Geneva produced the first experimental hypothyroidism in animals by thyroidectomy [277]. In 1873, Gull described a "cretinoid condition" which occurred chiefly in women [278]. Ord suggested the term myxedema for this condition in 1878 because of the large amount of mucin in the skin [279]. Reverdin [280] and Kocher [281] showed that myxedema occurs after removal of the thyroids. Bettancourt and Serrano [282] and Murray [283] showed that the administration of thyroid extract cured myxedema. Cretinism and myxedema were thus shown to be manifestations of thyroid hypofunction.

At the same time observations of hyperfunction of the thyroid were made. In 1835, Graves [284] described a disease in England characterized by goiter, tachycardia, and exophthalmos. Basedow [285] described this condition in 1840 in the German literature in Merseburg; hence, the name "Merseburger triad." The resulting disorder, hyperthyroidism, is known as "Morbus Basedow" or Graves' disease.

Pathology of Hypothyroidism

Hypothyroidism results when there is insufficient secretion of hormone by the thyroid gland. The production and secretion of thyroid hormone can be reduced or interrupted by exogenous as well as endogenous factors. Among such factors are reduced iodine intake in iodine-poor geographical areas, disturbed thyroid function because of exogenous toxins, disturbance of the pituitary regulation of the synthesis of thyroid hormone or the circulation of iodine, and constitutional factors. Anatomically, there may be hereditary defects, atrophy, degeneration, hyperplasia, fibrosis, and cyst formation. Clinically, the forms of primary hypothyroidism include congenital hypothyroidism with endemic and sporadic cretinism, juvenile myxedema (hypothyroidism during the ages of growth) and classical myxedema (hypothyroidism in the adult). Distinguished from these are the cases of secondary hypothyroidism, which are due to pituitary insufficiency.

Endemic cretinism occurs as a result of iodine deficiency in areas of endemic colloid goiter. It is caused by deficient fetal development of the thyroid gland because of an iodine deficient diet in the mother. Usually, the fetal thyroid tissue first enlarges to

produce a goiter under the stimulation of the thyrotropic hormone. Later, with increasing peripheral requirements for thyroid hormone the goiter can become exhausted and atrophy as a result of the prolonged excessive stimulation by thyrotropic hormone.

Sporadic cretinism with or without goiter differs from this condition. Usually, goiter is absent in sporadic cretinism. For unknown reasons, the thyroid gland is atrophic or completely absent, perhaps because of developmental abnormalities. McGirr and Hutchinson suggest that there is deficient descent of the thyroid gland [286]. The authors also observed a child with non-goitrous sporadic cretinism who failed to respond adequately to dry thyroid but reacted to L-3', 3,5-triiodothyronine. In this case they postulated an inborn deficiency of iodine metabolism located not in the thyroid, but in peripheral tissue, where the deiodination of thyroxine to triiodothyronine was deficient [286 a]. Sporadic cretinism with goiter, on the other hand, is due to intra-thyroidal disturbance of hormonal synthesis. The deficient secretion of thyroid hormone causes increased secretion of thyrotropic hormone. The thyroid becomes enlarged (goiter), hyperactive, takes up abnormally large amounts of I^{131} , but secretes no hormone iodine or only an incomplete product. Stanbury and Hedge [246] for example observed that the thyroid gland of such patients takes up iodine, but cannot oxidize it, and the accumulated iodine can again be replaced by thiocyanate. There is probably a lack of the oxidizing enzymes of the gland. Other authors observed disturbances of the condensation reaction or of the dehalogenase. Different stages in the synthesis of thyroid hormone may be disturbed [287, 288]. This form of cretinism is usually familial so that some authors feel that it also represents an inborn error of synthesis of the hormone [287].

Pituitary hypothyroidism is discussed elsewhere (Simmond's and Sheehan's disorders) [288 a]. Partial or complete failure of the pituitary is followed by a deficiency of thyrotropic hormone and disturbance of the equilibrium between pituitary and thyroid [1].

Astwood and his coworkers discovered a goitrogenic agent, 1,5-vinyl-2-thio-oxazolidon, in cabbage [289], and thus pointed out the effect of goitrogenic dietary substances on thyroid function. This plant material inhibits the synthesis of thyroid hormone in the same way as therapeutic antithyroid agents (pp. 170, 205). However, the action may be noticeable only in the presence of a predominantly carbohydrate diet, for example in prisoners. Hypothyroidism may also develop following the therapeutic or accidental administration of goitrogenic substances such as paraaminosalicylic acid [289], thiocyanate [290], sulfonamides [291], thiouracil [292], 2-mercapto-imidazol [293], and resorcinol [294, 295].

Inflammation of the thyroid, thyroiditis, usually results in atrophy of the gland. The most common causes of hypothyroid states are the clinical use of x-ray or radioiodide, and operative removal of the thyroid gland. Partial resection is followed after a several years by degeneration and atrophy of the portion left behind. In many cases of hypothyroidism, the cause cannot be determined.

The changes in iodine circulation are caused by partial or complete failure of the thyroid glands. As a result, a test dose of radioiodide is not taken up by the gland but is excreted in the urine (Tables 48, 49; Fig. 50). The dose appears quantitatively in the urine within a few days, although with the failure of the thyroid, the renal clearance of inorganic iodine falls to 18 cc/min, as compared with the normal 34 cc/min [256]. Production of thyroid hormone is partially or completely reduced. Little or no protein-bound iodine is present in the serum: $< 3 \gamma\%$ in 14 myxedema patients studied

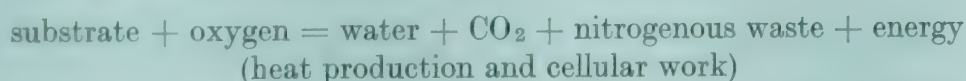
by Riggs, in contrast to the normal 3.5 to 7.0 % [256]. The fall of protein-bound iodine had been noted as long ago as 1925 by Veil and Sturm [272]. The reduced plasma hormone level is indicated by the reduced ratio of protein-bound iodine to total iodine.

The effect of thyrotropic hormone on the circulation of iodine in hypothyroidism permits differentiation of different types of hypothyroidism. In "myxedema" due to deficiency of thyrotropic hormone (pituitary hypothyroidism), the administration of thyrotropic hormone results in increased flow of blood through the thyroid gland, increased uptake of radioactive iodine by the thyroid, elevation of protein-bound iodine in the blood, and reduction of urinary excretion of radioactive iodine. The same results obtain when the thyroid is inhibited by iodide. In cases of primary hypothyroidism, due to anatomic changes or the use of anti-thyroid drugs, thyrotropic hormone has no effect.

In primary myxedema with normal hypophysis, large amounts of thyrotropic hormone are present in the urine [296], because of increased secretion of thyrotropic hormone by the pituitary gland. In hypothyroidism due to pituitary disease, however, thyrotropic hormone is diminished, so that none is present in the urine. The amount of thyrotropic hormone can be measured by biological means [296].

Deficiency of thyroid hormone in the body leads to reduced oxygen utilization throughout the body, including the musculature, heart, kidneys, lungs, skin, bone marrow, and liver. The percentual effect on each tissue is not known. It is not known if the sex organs, the lymphatic system, and the brain (see p. 195) likewise show reduced oxygen utilization in the presence of thyroid deficiency. Some authors believe that these tissues are not affected by thyroid hormone [262]. In calculations of oxidative processes, these tissues actually play only a small part, so that they have virtually no effect on basal metabolism.

The relationship between oxygen utilization and energy production is expressed in the following equation of cellular respiration:



Thyroid hormone regulates the physiological coupling of energy production and energy utilization and controls the magnitude of total metabolism. In malfunction of the thyroid, the ratio of these events within the cells becomes abnormal. In hypothyroidism, the total metabolism is lowered, and the same cellular processes require less oxygen than under normal conditions. Presumably an excess of available energy for cellular work is present. Thus, the patient with myxedema needs 10% less oxygen to walk than the normal person [297]. Not all authors agree with these statements, however. Thus, Boothby and Sandiford found that the utilization of oxygen was normal (Table 12).

In myxedema, the basal metabolic rate is reduced. The reduction is the same in myxedema and pituitary hypothyroidism. In complete absence of hormone synthesis, especially in congenital absence of the thyroid, the BMR may be as low as -- 50%; that is, the resting oxygen requirement is half the normal. However, the level of basal metabolism is dependent on other factors besides thyroid hormone, as shown by the basal metabolic rate in various conditions which have nothing to do with the thyroid. Reduction to as low as -- 35% may be seen in starvation, Addison's disease, and severe depressions. The reduction of basal metabolism may be used diagnostically only in combination with other data (blood iodine, tissue function, body state, anatomic changes, general status of the patient).

Reductions proportional to the reduction of oxygen utilization also occur in the utilization of substrate, production of heat, loss of water, excretion of carbon dioxide, and excretion of nitrogen. The reduced utilization of substrate is the result of reduced caloric requirement of the patient. The reduced production of heat is equalized by diminished flow of blood through the skin (i. e., by improved insulation). Most patients with myxedema are very sensitive to the cold, and the normal rectal temperature may be less than 97° F.

Cardiac muscle uses less oxygen and substrate both in vivo and in vitro, yet cardiac insufficiency does not follow. Cardiac failure is rare and is seen only in the late stages of the disease. The heart shows bradycardia, reduced stroke volume, low blood pressure, and low minute volume. Scheinberg et al. [298] studied 7 myxedematous patients by means of the Fick method. The reduction of oxygen utilization averaged 37%, and the corresponding reduction of minute volume index was 47% (normal 3.54 liters/min per square meter of surface area; myxedema 1.89 liters/min per square meter of surface area). The pulse rate ranged from 48 to 79 per minute and the median arterial blood pressure was from 70 to 114 mm Hg.

In the peripheral circulation, there is an elevation of the peripheral resistance with reduced blood flow. In Scheinberg's patients, the resistance of the cerebral blood vessels was raised from the normal 1.31 to a level of 2.5 mm Hg per cc blood per 100 grams brain per minute. In addition, the cerebral blood flow fell from the normal 65 ± 2.1 cc per 100 grams brain per minute to a level of 40 ± 3.7 cc, in good correlation with the fall of the basal metabolic rate [298]. Reduced blood flow in the skin has been reported by many authors [1].

Pulmonary function is also reduced. Bansi reports a reduction of the alveolar carbon dioxide tension with reduced carbon dioxide content of the exhaled air (2.5 to 3%, normal 3.4%). The saturation of the arterial blood of Scheinberg's patients was reduced (70 to 97%, with an average of 86%), while Bansi's figures were 89 to 93% arterial saturation [1].

Kidney function is reduced, as shown by the iodine clearance. Neurologically, the reflexes are reduced.

With regard to the pathogenesis of the manifestations of hypothyroidism, it is important to know if the transport function of the blood and circulation is adequate to supply oxygen and substrate (especially glucose) to the peripheral tissues and to remove carbon dioxide and nitrogenous waste. When the supply of oxygen is insufficient – chronic hypoxia – the reduction of metabolism and the reduced oxygen utilization may be the result of the oxygen deficiency. The question is especially important with regard to the brain, for, in recent years, there has been some question whether the metabolism of the brain is under the control of thyroid hormone [262]. If such control exists, the cerebral symptoms of myxedema could be explained by oxygen deficiency. The question has not yet been answered.

The supply of glucose to the tissues depends on the level of blood sugar. The blood sugar in hypothyroidism tends to be low normal. Five patients with myxedema studied by Crawford [299] showed reduced utilization of glucose after the intravenous injection of 0.5 grams of glucose per kg body weight. Scheinberg [298] found reduced utilization of glucose by the brain. The difference between arterial and venous glucose, normally 9.9 mg%, is not significantly elevated in myxedema (11.3 mg%).

The dietary intake of nitrogen is also reduced, because of the reduced intake of food, so that less nitrogen is excreted. The nitrogen balance can be made normal by administration of 45 grams of protein. It is not known if nitrogen metabolism is also

reduced as an expression of the dynamic equilibrium of body proteins, but this seems likely. The rate of protein synthesis, measured by the N^{15} -glycine technique, was found to be decreased in six patients with myxedema [299 a]. The specific dynamic action of the proteins is delayed or absent; this may also be a sign of slow utilization of nitrogen.

The level of serum proteins is somewhat higher than normal. Lichtwitz [300] described a case of hypothyroidism with a plasma protein level of 9.2 grams $\%$. The serum globulin may be elevated and the serum albumin reduced. The protein of the cerebrospinal fluid is also elevated (28 to 221 mg $\%$, with normal 20–40 mg $\%$) [301]. The extracellular fluid, in contrast to that of cardiac or renal edema, is rich in protein. Boothby's calculation gave a protein content of 2 grams $\%$ [302]. The level of amino acids in the serum after thyroidectomy is normal, but the level in advanced myxedema is not known. Immediately after the injection of thyroid hormone there is an elevation of nonprotein nitrogen in the serum [303] and in hyperthyroidism there is an increased excretion of amino acids in the urine. The amino acid excretion in the urine in myxedema is not known, and the concentration of free amino acids and protein in the intracellular space is also not known.

The myxedema protein of the tissues carries large amounts of water with it, hence the development of edema. Edema occurs in the subcutaneous fat, the tissue spaces of the skeletal muscles and the heart, and in the connective tissue. The edema fluid, like all extracellular fluids, contains sodium as an important cation. The results of this binding of water are disturbances of the water balance and distribution of the body. As myxedema protein is deposited in the extracellular space, it draws plasma water along with it and the plasma volume is reduced. The average plasma volume in nine patients with myxedema was 35.4 cc per kilogram body weight, as contrasted with the normal 43 cc [305]. The protein-rich extracellular fluid produces a new Donnan equilibrium which affects diffusion, exchange, filtration, and secretion. The excretion of water is markedly reduced because of the binding of water in the edematous fluid. In studies in thyroidectomized dogs, Eppinger [306] found that, three hours after the administration of 300 cc of water, only 91 cc was excreted, as contrasted with the normal 184 cc.

The nitrogen of myxedema protein and the excess extracellular fluid are excreted after treatment with thyroid hormone. Such excretion is a criterion of effective therapy (p. 164). However, there is no parallel between the excretion of nitrogen and that of water.

There is a tendency to increased synthesis of lipids. High serum cholesterol values were found by Epstein and Lande [307] in 1922, and elevated serum cholesterol is of some diagnostic value in hypothyroidism. The level of phospholipids also rises, more or less proportional to the rise of cholesterol. Neutral fats and fatty acids rise less constantly [308].

The myxedematous disturbance of cellular respiration is accompanied by shifts of the creatine-creatinine equilibrium of the body (p. 115). In cretinism and juvenile myxedema, the excretion of creatine falls from 150–200 mg day to 10–40 mg day. The excretion of creatinine, however, rises. The oral administration of 1 gram of creatine to hypothyroid patients results in retention of 86 to 95% of the dose (normal, 70 to 80%) [309] (Table 47). There is no change in the excretion of creatinine. Wang [311] showed an increased tissue concentration of creatine and creatine phosphate in hypothyroid rabbits. Approximately 50% of tissue creatine is present as creatine phosphate. Since high energy phosphate compounds are in equilibrium with each

Table 47. Creatine Tolerance in Hypothyroid Children [310]. (Mean values)

Cases	Number of cases	Age in years	Daily excretion (mg)	Average Excretion after a 3 gram creatine loading test	% retention of creatine
Normal Girls	18	9.4	185	1.787	43.2
Boys	13	8.8	142	1.494	54.6
Hypothyroid children	5	10.4	65	1.200	62.2

other, it may be concluded that the hypothyroid rabbit, despite reduced basal metabolism, synthesizes more high-energy phosphate than the normal.

Clinical Hypothyroidism

Adult hypothyroidism occurs chiefly in women. In general, hypothyroidism is less common than hyperthyroidism. Means [312] found 59 cases of myxedema in Boston from 1925 to 1935, a ratio of 1 case of myxedema to 8 cases of hyperthyroidism. The same data apply in Europe.

The development of the clinical picture is gradual and nonspecific. The chief symptoms are insomnia, digestive difficulties, skin changes (loss of hair and changes in the nails), changes of the voice, and generalized fatigue and muscular pains. There are disturbances in menstruation and loss of vitality. Psychiatric symptoms may be prominent.

Patients with myxedema are sluggish, dull, indifferent; but can act normally and show signs of intelligence. Most patients show premature aging and act senile or infantile. Signs of irritability may be present. Impotence is present, the menopause occurs early, and menses are irregular or absent. Bürger [313] and Bansi [1] note a parallel to the age incidence of arteriosclerosis. The motion of the body is sluggish, the mind is sluggish, the tongue is thick and heavy, and speech is thick and difficult. The voice, especially in women, is coarse and husky. The face is bloated and the body fat. The changes in the skin occur chiefly in the face and at the distal portions of the extremities. The skin is cold and dry, scaly, and pale (at first pallid, later yellowish, still later cyanotic). The skin is soft and doughy, and there is no pitting after pressure. The appendages show trophic changes. The nails become cracked and blunt; the hair becomes brittle and dry. The secretion of sweat stops, even after pilocarpine.

In spontaneous, sporadic myxedema, the thyroid gland is palpable. In iodine-deficient areas, goiter is usually present. In chronic thyroiditis, diffuse enlargement of the thyroid gland is present. The heart shows dilatation, and pericardial effusion occurs sooner or later during the disease. The blood pressure is low and the pulse slow. The electrocardiogram shows a characteristic reduction of voltage with flat T- and P-waves. The peripheral blood flow is reduced, the peripheral resistance is elevated, and the minute volume of the heart is reduced.

The blood usually shows a hypochromic or normochromic anemia, although macrocytic anemia without hyperbilirubinemia may be present. The serum iron is normal.

There is impaired gastrointestinal motility, and intestinal atony often occurs. The intestinal secretions are reduced, the saliva is moderately reduced and thickened, and gastric hypoacidity is present. In the absence of previous diseases or cardiac failure,

the liver is not enlarged and liver function tests are normal. Renal function as measured by urea clearance or iodide clearance is reduced. Water balance is altered as already described.

Hypothyroidism, untreated, is slowly progressive. The basal metabolism gradually falls to levels below -40% . The body weight increases and the body temperature falls. This level of metabolism may be present for years. Ultimately, the patient passes into a vegetative state without any activity. Anorexia is followed by gradual loss of body stores, and myxedematous cachexia may follow. Intercurrent infection may occur and lead to coma and death.

The course and symptomatology in a given patient depend on the status of the body at the onset of the disorder. Myxedema may thus occur after puberty and at the menopause; although the basic abnormalities are the same, the two types of myxedema differ from each other. Of great importance, perhaps, is the amount of extrathyroidal hormone synthesis in the body. Age and the presence of other diseases are especially important. Postoperative myxedema is frequently accompanied by hypoparathyroidism, with the clinical findings of both deficiencies. As a result, the metabolism of electrolytes is abnormal, and tetany may occur. Secondary hypothyroidism due to pituitary disease differs from myxedema by the presence of signs of other endocrine abnormalities and the lack of the typical skin manifestations [288a].

In the full-blown picture, the diagnosis of myxedema is relatively simple because of the external appearance of the patient, the characteristic speech and the changes in the skin and hair. An old photograph may help by showing the changed appearance of the patient. Psychic changes accompany the physical ones. Bradycardia, intestinal disturbances, anemia, sensitivity to cold, low basal metabolic rate, body weight, high serum cholesterol, and abnormal protein-bound iodine help establish the diagnosis. Radioactive iodine tests may be diagnostic.

Myxedema must be differentiated from secondary hypothyroidism. In pituitary hypothyroidism, x-ray of the sella turcica and tests of other pituitary functions are abnormal (adrenal cortex, pancreas, sex glands).

Most of the signs of myxedema are nonspecific. The basal metabolic rate may be decreased in various other conditions, such as Cushing's disease, acromegaly, Addison's disease, and nephrosis. The serum cholesterol may also be high in diabetes mellitus, nephrotic syndrome, and essential hypercholesterolemia, but it is normal in Addison's disease and Simmond's disease. Edema may be seen in renal disease, congestive heart failure and starvation (protein- and vitamin-deficiency). The pericardial effusion of late myxedema resembles that seen in uremia.

If diagnostic methods cannot be completely performed, the diagnosis may be made by a therapeutic test with adequate amounts of thyroid hormone. A maintenance dose is necessary to maintain the normal state.

According to the older data of Thompson et al [314] (1935), the body contains a total of 10 to 14 mg of thyroxin. More recent studies by Riggs [256] give a value of 50 % of protein-bound iodine per liter of cell fluid in the adult. For a 70 kg man, this amounts to 2.5 mg of protein-bound iodine (3.8 mg thyroxin or 4.3 mg triiodothyronine). The difference between the two sets of data is due to the fact that protein-bound iodine of the thyroid is not included in the more recent data of Riggs. The healthy body requires, according to the normal rate of secretion of thyroid, approximately 110 % of thyroid hormone, calculated as thyroxin, per day. The management of myxedema must therefore replace the hormone which is lacking (approximately 2.5 mg protein-bound iodine) and must then continue to substitute for the absent

hormone. The hormone requirement of young people is somewhat greater than that of older.

The patient with myxedema is exceedingly sensitive to thyroid hormone. He utilizes administered hormone rapidly and optimally, and the drug must be given with great care. In high grade myxedema, because of absorptive disturbances, much of the hormone given by mouth is lost. Following intravenous administration, the hormone is rapidly distributed throughout the body. However, the danger of destruction of the hormone, especially by the liver, is great [315], for unphysiologically high serum concentrations occur following intravenous administration. For this reason, it is best to administer the drug intramuscularly at first, or to give oral doses sufficiently large to compensate for the loss in the bowel.

Both thyroid hormones, thyroxin and triiodothyronine, are qualitatively of equal value. Quantitatively, triiodothyronine is superior. Both substances can be given by mouth, and triiodothyronine is better absorbed than the sodium L-thyroxin which is commonly used. Gross, Pitt-Rivers, and Trotter [258] found that their 3 patients required not more than 5 mg of triiodothyronine to become euthyroid. The basal metabolic rate was raised from -40% to normal and remained normal on a maintenance dose of 80 μ of triiodothyronine daily. Rigg's value of 4.3 mg triiodothyronine agrees well with these findings. Thyroxin may be given in essentially the same amounts. These values, which differ from those formerly recommended, reflect the manner of administration of the hormone and its destruction in the liver.

The daily dose is divided into several doses of 20 to 25 μ of triiodothyronine per day. The initial dose of 20 μ per day is given with caution for 3 to 5 days, then raised in accordance with the clinical response until a maintenance level is reached. In one case, for example, Gross et al. gave 20 μ daily for the first 9 days, then 40 μ daily for 5 days, then 80 μ daily intramuscularly, and attained a normal BMR after 22 days. When thyroxin is used, the beginning dose is 50 μ per day, and the oral maintenance dose is 0.1 to 1.0 mg daily [378, 379].

The criterion of successful therapy is, above all, clinical improvement. There is prompt improvement of the skin, the voice, speech, and cardiac symptoms and signs. Progressive improvement may be followed semi-quantitatively by the changes of the excretion of water (loss of weight), the nitrogen excretion, the blood cholesterol, and the basal metabolic rate. The response of cholesterol and BMR is much slower than that of water excretion (diuresis) and of general clinical improvement. The best sign that a maintenance dose has been reached is the general well-being of the patient. Especially in older patients, the euthyroid state may not be reached for several months. The patient must be watched carefully during this time and during the period of maintenance therapy. The patient has a tendency to forget to take the drug and may therefore rapidly become myxedematous once again.

The types of thyroid preparations and artificially iodinated proteins (e. g., iodinated casein) are of some interest. The protein-bound iodine of these substances consists of triiodothyronine and thyroxin, as well as non-hormonal iodine (monoiodotyrosine, diiodotyrosine, etc.). The amounts of these substances in different preparations is not the same. Since only hormone iodine is effective in myxedema, the effect of different preparations is not the same if one considers the total iodine content as the basis of dosage. The content of hormone iodine decides the dose, but this value is not generally given for the usual preparations. The effect of the preparations in biological tests is roughly proportional to the content of hormone iodine, and preparations may be standardized on this basis. Metabolic units or axolotl units are used: 1 metabolic

unit equals 17.5 alcohol units and is equivalent to the activity of 5 γ of thyroxin given subcutaneously [4]. Preparations of desiccated thyroid have the advantage over the hormone that they act slowly, like depot preparations, for the body must make free hormone from the protein-bound form. The use of desiccated thyroid is therefore more physiological. In the United States Pharmacopeia, preparations of thyroid must contain 0.17 to 0.23 percent of iodine in the form peculiar to that found in the thyroid gland.

In treating myxedema, it is necessary to pay attention not only to administration of thyroid hormone but also to an adequate caloric diet and adequate amounts of vitamins. In addition, any other coexisting diseases must also be treated. Pituitary hypothyroidism is treated with thyrotropic hormone, but only when the thyroid is still potentially functional. If this is no longer the case, thyroid hormone must be given just as in primary hypothyroidism.

In the growing organism, the signs of hypothyroidism are essentially the same as in the adult. There are special effects, however, of the lack of thyroid hormone on the growing organism. There are disturbed growth and impairment of differentiation. There is impaired formation of the growth centers of the carpal bones, of longitudinal growth of bones, of the eruption of teeth; closure of the cranial sutures is delayed. The development of hair, sexual organs, and sensory organs (especially the inner ear) is delayed or even absent. The nail bed capillaries remain undeveloped [316]. The mental development of infants is retarded. There is reduction of general vitality and strength. The muscles show pronounced hypotonicity, and posture becomes poor. The other findings are the same as in the adult, including retention of water, changes in the skin and its appendages, husky voice, and constipation.

It is difficult to measure the basal metabolic rate of infants and children. The serum cholesterol is elevated, up to 300 to 800 mg%. The serum inorganic phosphate is lowered to 1 to 2 mg%. The creatinuria which occurs physiologically in children is absent. The typical changes of myxedema are found in the electrocardiogram. Early diagnosis is aided by the use of radioactive iodine together with thyrotropic hormone, which shows hypothyroid excretion and retention of iodine [259].

Proper and prompt diagnosis is necessary for the proper development of the child. The fate of the child may depend on the promptness of management. Disturbances of development of the nervous system may already be irreversible even with the use of large doses of hormone. The principles of treatment are the same as in the adult, although the maintenance dose in childhood is somewhat higher. On proper dosage depend normal growth, normal formation of teeth and bones, and normal development of the sexual organs and of mentality. Treatment is followed by diuresis and weight loss, and later by increase in weight, as the hormonal balance of the body is restored to normal.

Pathology of Hyperthyroidism

Hyperthyroidism includes those states characterized by overproduction and oversecretion of the thyroid hormones. Several forms are distinguished clinically, including severe thyrotoxicosis and thyroid storm as well as milder forms of hyperthyroidism. Exact separation into mild, severe, and very severe forms is not possible, and the general term "hyperthyroidism" or "thyrotoxicosis" is preferred.

Overproduction of thyroid hormone may be due to diffuse hyperplasia (Basedow) or toxic nodular goiter. In diffuse hyperplasia, the entire gland takes part in the ex-

Table 48. The circulation of iodine in normal and pathological states
(Mean values, from Horst [259])

	Normal	Hyperfunction	Hypofunction
Iodide Phase			
Thyroid Gland			
Iodide clearance	7-42 cc	85-500 cc	1- 4 cc
Uptake of a test dose of radioactive iodine			
After 2 hours	8-25%	25-70%	0- 5%
After 24 hours	10-45%	45-90%	0-10%
Urine			
Excretion of a test dose			
0-24 hours	28-76%	6-27%	60-90%
24-28 hours	2- 9%	0- 3%	8% and more
4- 8 hours	5-20%	0.2- 5%	8% and more
Thyroxin Phase			
Conversion Rate	13-45%	45-96%	—
Serum level of radioactive iodine after 48 hours:			
a) percent of dose per liter	0.0-0.5%	0.5- 5%	—
b) percent of (a) which is protein-bound	15-70(?)%	70-100%	—

cessive hormone production. The gland seems no longer to be under any sort of control. The rate of hormone production is pathologically increased. Toxic adenoma produces and secretes in the same manner as a functional pancreatic adenoma: it is an overactive zone within an otherwise normal gland, and produces uncontrolled amounts of thyroid hormone. This is an autonomous pathological growth which can be promoted and, perhaps, stimulated by excessive administration of iodine or thyrotropic hormone from the outside.

The actual causes of overproduction of thyroid hormone are quite unknown. Normally, the amount of hormone formed and secreted is determined by the requirements of the peripheral tissues in the same manner as the requirement of oxygen [256]. In hyperthyroidism, it is not known whether the peripheral tissues primarily require pathological amounts of hormone or are pathologically sensitive to the hormone, whether the thyroid in itself produces increased amounts of hormone, or whether the pituitary or other controlling cerebral centers stimulate the thyroid to excessive activity. The role of the thyroid-stimulating hormone is still under discussion [7 a], but there is some indication that the activity of this hormone is increased in Graves' disease [316 a]. The clinical picture of hyperthyroidism can be reproduced experimentally by administration of thyroid hormone or thyrotropic hormone. Möbius believed hyperthyroidism to be of thyrogenic origin [317], but his theory is doubted because experimental hyperthyroidism does not reproduce the entire symptomatology of clinical hyperthyroidism. Charcot championed a neurogenic theory [318]. However, this theory is not acceptable because the fundamental sign of hyperthyroidism, elevation of metabolic rate, can be produced experimentally even after denervation of the thyroid gland.

The pathogenesis of the symptomatology of hyperthyroidism is the continuous overloading of the body with thyroid hormone. The increased concentration of hormone leads to pathological metabolism within the body cells, to changes of equilibrium, and to pathological states of cells and tissues.

The characteristic findings of increased circulation of iodine are listed in Tables 48 and 49 and in Fig. 50. The total metabolism of the thyroid gland is increased up to ten-fold. The thyroid itself shows hypertrophy and congestion. These facts are reflected by the so-called "thyroid clearance" of Myant and Pochin [319]: for the normal thyroid, the value is 16 to 25 cc of plasma/min; for pathological thyroids, it averaged 496 cc/min in 11 cases, with a maximum of 1300 cc/min in one case. The hypertrophy thus affects not only the amount of thyroid tissue itself, but also the amount of functional surface area. The gland is devoid of colloid and the content of total iodine, as well as the proportion of thyroxine iodine to total iodine, are markedly lowered. In eleven patients studied by Wilson and Kendall [320], the total iodine content of the gland averaged 0.6 mg/gram dry weight, as compared with the normal value of 1.8 mg/gram. Of the total iodine, 16% was present as thyroxine, as compared with the normal 25% [321]. The thyroid apparently takes up inorganic iodine from the blood stream and immediately secretes synthesized hormone, without any storage, back into the blood.

Testing with radioactive iodine (I^{131}) shows an increased concentration of inorganic radio-iodine within the thyroid gland, plus an elevated rate of secretion of thyroxine into the bloodstream. The normal uptake of radioactivity 24 hours following a test dose is 10 to 45%; in hyperthyroidism, the uptake measures 45 to 90% [259]. The rate of secretion can be determined from the percentage of radioactivity detectable in the blood as thyroxine iodine and the time of its appearance in the serum. Horst is especially concerned with the so-called "thyroxine phase" or "hormone phase" in clinical diagnosis. These studies are summarized in Figures 46 and 47 and Table 48.

The serum hormonal iodine rises in proportion to the increased secretion by the gland. As long ago as 1927, Veil and Sturm [272] pointed out that the blood iodine is elevated in hyperthyroidism. Modern methods of determination give normal values of 3.5 to 7.0 $\gamma\%$; in hyperthyroidism, the values are 8 to 48 $\gamma\%$. Hormonal iodine is distributed to the peripheral tissue spaces. Any excess is lost in organic form in the gastrointestinal tract. The peripheral tissues rapidly catabolize hormone iodine into inorganic iodine (iodide). Some of the iodide is again taken up by the thyroid gland; some is excreted in the kidneys and the lungs. The renal clearance of iodide is normal, but the excretion of inorganic iodine (iodide) is increased. Depending on the severity of the disorder, values as high as 770 γ of iodide may be excreted daily. The patient of Figure 50 excreted 199 γ /day. The excretion results in a slightly negative iodine balance, even if the amount of iodine taken in in the diet is increased. As a result, severe iodine deficiency may occur as the disease progresses. If, instead of these studies, the excretion of radio-iodine following a test dose is measured, there is first a retention of radioactivity within the body because of the uptake of radio-iodine by the hyperactive thyroid gland. The excretion of radio-iodine therefore reflects the activity of the thyroid glands, and is thus useful in diagnosis.

The iodide space of the body, which corresponds to the extracellular space, is slightly increased, while the hormone-iodine space, which corresponds to the intracellular space, is normal. The increased iodine turnover of the thyroid gland leads to a marked reduction of its hormone content. The distribution of hormone iodine between thyroid and peripheral tissue is thus shifted in favor of the periphery. Normally, only 15% of the total hormone iodine is found in the peripheral tissue; in hyperthyroidism, this figure becomes as high as 61%, with the remainder within the thyroid gland itself. Riggs gives a value for the normal of 400 mg of organic iodine per kg of thyroid [259]; in hyperthyroidism, the corresponding value is 91.7 mg. Gutman and

his coworkers found similar results [322]. The fact that the normal thyroid gland has a tremendous avidity for iodine and stores 85% of the total hormone iodine of the body prevents a deficiency of hormone within the body, and protects against exogenous deficiency of iodine. A continuous, steady secretion of thyroid hormone is thus guaranteed. These facts no longer hold in thyrotoxicosis and there is practically no storage of hormone at all. In clinical practice, thyrotoxicosis may be followed by myxedema: this transformation may be related to the deficient iodine reserves within the thyroid gland.

Müller was the first to show the increased metabolic rate in hyperthyroidism [323], and his data show that the thyroid hormones affect the level of metabolism. The

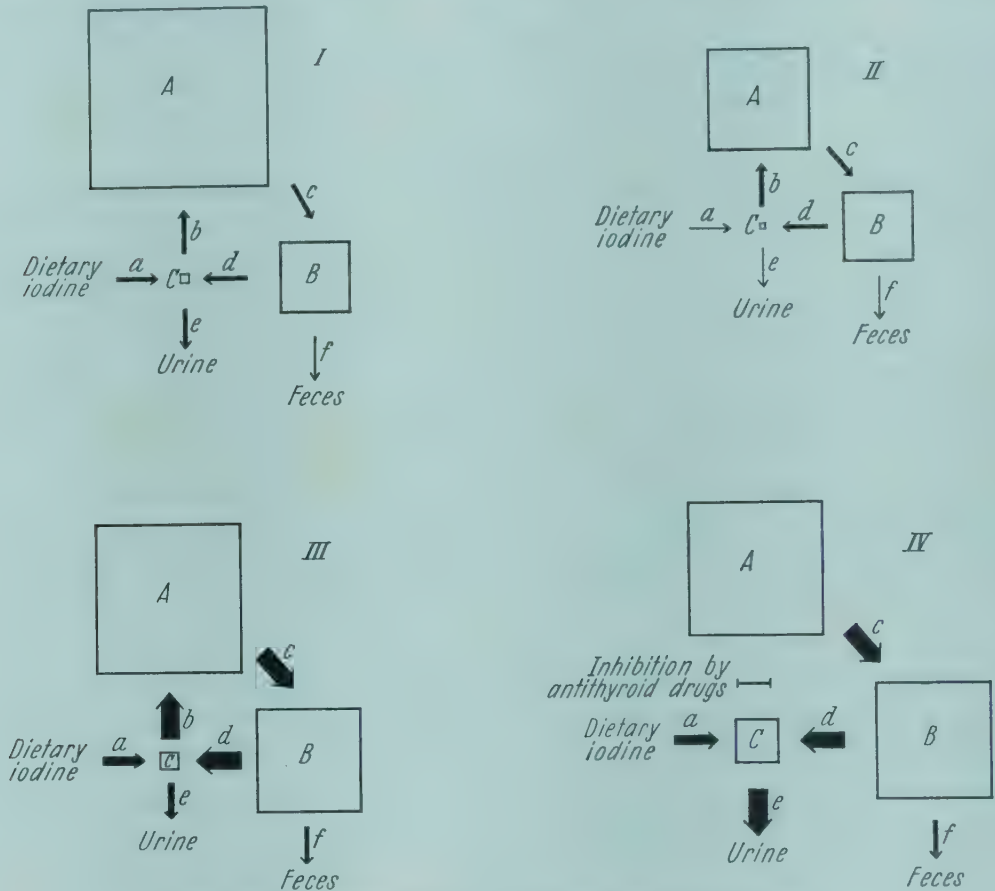


Fig. 50. The Metabolism of Iodine. (See Table 49.)

- A-C: Steady-state concentrations. The size of the squares is in proportion to the amount of iodine
- A: organic bound iodine in the thyroid gland
- B: organic bound iodine in the periphery, including the blood (hormone iodine)
- C: total inorganic iodine in the body
- a-f: Daily turnover. The width of the arrows is approximately proportional to the turnover.
- a: iodine intake in food and drink
- b: uptake of inorganic iodine by the thyroid
- c: amount of hormone secreted
- d: hormone iodine minus excreted organic iodine (chiefly fecal). That is, $d = c - f =$ inorganic iodine liberated from organic binding.
- e: excretion of iodine in the urine
- f: excretion of iodine in the feces
- II. Normal individual with optimal intake of iodine
- II. Normal individual with iodine deficiency
- III. Hyperthyroidism
- IV. Hyperthyroidism under treatment

See also Table 49.

pathogenesis of this elevated metabolism lies in the increased cellular oxidation. The hyperthyroid body utilizes substrate and oxygen more rapidly than normal and produces carbon dioxide, water, and nitrogenous waste more rapidly. This statement can be verified by measuring the individual components of the general equation of cellular respiration:

$$\text{substrate} + \text{oxygen} \rightarrow \text{carbon dioxide} + \text{water} + \text{nitrogenous waste products} + \text{energy (heat and cellular work)}.$$

The total metabolism is increased. The amount of oxygen taken up or carbon dioxide given off under basal conditions is a measure of the severity of the disease (Magnus-Lavy [51]). The increase in metabolism is the result of increased amounts of thyroid hormone in the body and can be reproduced experimentally by feeding thyroid

Table 49. Normal and Pathological Iodine Metabolism. Modified from [256]

- I. Normal person with optimal intake of dietary iodine.
- II. Normal person with deficient dietary iodine but in iodine equilibrium. The body spares the iodine ingested by marked reduction of urinary excretion and by reduction of the amount of inorganic iodine stores. As a result, the thyroid undergoes marked enlargement under the influence of thyrotropic hormone.
- III. Moderately severe hyperthyroidism. Increased turnover of iodine with a slight increase in the excretion of iodine in the urine and the stool. Increased intake of food results in increased intake of iodine.
- IV. Hyperthyroidism shortly after the start of treatment with antithyroid drugs. Marked increase of iodine excretion in the urine.
- V. Hypothyroidism and athyroidism.

	I	II	III	IV	V
Dietary iodine (γ/day) (a)*	150	15	250	250	—
Thyroid uptake (γ/day) (b)*	70	70	597	—	—
Hormone secreted (γ/day) (c)*	70	70	597	597	—
Weight of thyroid (grams)	20	120	60	60	—
Thyroid clearance (cc/min)	16	259	100	—	—
Thyroid uptake of inorganic iodine (% of tracer dose)	30	88	75	—	—
Renal clearance of inorganic iodine (cc/min)	33	33	33	33	17.9
Urinary excretion of inorganic iodine (γ/day) (e)*	144	9	199	796	—
Serum hormone iodine (γ %)	6-8	6-8	8-25	8-25	1-2
Fecal iodine (γ/day) (f)*	6	6	51	51	—
Basal metabolism	normal	normal	high	high	low
Serum cholesterol (mg%)	120-270	120-270	50-140	50-140	220-500
Creatinuria	normal	normal	+	+	—
Thyrotropic hormone in the urine	normal	in-creased	in-creased? or normal	in-creased	in-creased or nil

* See Figure 50.

extract or purified thyroid hormone. The increase can be observed in isolated tissues, in tissue homogenates, and in the mitochondrial fraction of the cells under hyperthyroid conditions. Thus, thyroid hormone causes elevation of the oxygen utilization of the splanchnic tissue, including the liver [324], of the isolated perfused hind-leg of a dog [325], and of the isolated functioning heart of a rat [326]. The same reaction is present in the liver, skeletal muscle, cardiac muscle, diaphragm, skin, bone marrow, kidneys, and lungs of different laboratory animals under hyperthyroid conditions.

However, it is not known for certain whether the spleen, the lymphatic system, the sex organs, and the nervous system also develop increased utilization of oxygen [262]. The nervous system is especially important in this regard. Scheinberg [327] and Kety [328] studied hyperthyroid patients by the method of Kety and Schmidt and found that the utilization of oxygen by the cerebrum is normal and does not parallel the increase in the basal metabolism. Barker [262] and Hoexter [329] found no increase of oxygen utilization in brain preparations of hyperthyroid rats. (On the other hand, brain preparations taken from hypothyroid animals show reduced oxygen utilization, which can be returned to normal by the addition of thyroid hormone.) Scheinberg [327] believes that the brain normally utilizes a maximum amount of oxygen and that this amount cannot be increased any further, so that no increase can be anticipated in hyperthyroidism. Against this concept, however, is the known fact that epinephrine and muscular effort *in vivo* both increase the utilization of oxygen by the brain [330].

The oxygen utilization of the peripheral nervous system has been poorly studied. Thyroxin has been found to increase the oxygen utilization of the vagus nerve in the dog. Netter calculated that maximal stimulation of human nerves would increase the daily caloric turnover by only 7 calories as compared to normal [17]. The state of irritability of the nervous system therefore makes no contribution to the elevation of the basal metabolism. The elevation of oxygen utilization of the various tissues of the body is not dependent on the nervous system, as evidenced by the fact that the oxygen utilization remains elevated after denervation. The utilization of oxygen and the formation of carbon dioxide continue to be increased in various animals after section of the cervical or thoracic cord or of the nerves of the arms and legs, after splanchnicectomy, vagotomy, or extirpation of the stellate ganglion. As long ago as 1897, Georgiewsky showed that even the denervated heart shows tachycardia after thyroxin; this tachycardia is proportional to the utilization of oxygen and persists even after isolation [331]. The effect of thyroid hormone persisted after damage to the bundle of His or the sinus node. The high oxygen utilization of hyperthyroid cells can be reduced or completely stopped by poisoning Warburg's respiratory enzyme. This fact shows that the oxygen utilization in hyperthyroidism is a reflection of the increased cellular respiration.

The increased oxygen turnover is not dependent on the diet. It continues to be present in hunger, in which case the body oxidizes increased amounts of its own tissues. Friedrich von Müller observed a negative nitrogen balance in hyperthyroidism [323]. However, this does not always occur, but is dependent on the amount of food taken in as well as the size of the carbohydrate and fat depots. Lauter [332] and Krauss [333] showed that protein is metabolized only to the degree that it satisfies the increased energy requirements. Krauss obtained a normal nitrogen minimum in hyperthyroid patients by feeding very high caloric diets rich in fat and carbohydrate showing that the nitrogen requirement in hyperthyroidism is not increased if sufficient fat and carbohydrate are present to serve as the substrate for oxidative processes [333].

When the diet is normal, carbohydrates (as glycogen) and fat are stored in depot for subsequent oxidation as needed. In starvation, the body first uses carbohydrate for oxidation, then fat and protein. The same is true in hyperthyroidism; the three foodstuffs are involved in oxidative processes depending on the amount of food taken in, the size of the depots of carbohydrate and fat, and the degree of elevation of metabolism (i. e., the severity of the disease).

The amounts of the different foodstuffs actually involved in oxidation can be determined from the respiratory quotient, nitrogen balance, and creatine excretion. In hyperthyroidism, Bansi found a respiratory quotient of 0.787 to 0.706, indicating oxidation of fat and protein [1,334]. The amount of creatine excreted is in direct ratio to the size of the glycogen depots in the muscles and the liver. Brentano's experimental studies in animals showed that, in various conditions of starvation and in hyperthyroidism, creatinuria always appears when the glycogen depots are exhausted. Creatinuria occurs in all cases of hyperthyroidism when the caloric intake is insufficient. Other experiments have shown that the glycogen depots are rapidly exhausted in hyperthyroidism.

Using Bansi's data, it can be calculated that, if a normal amount of protein is oxidized, the proportion of carbohydrate burned is 30%, and that of fat is 70%. If the nitrogen balance is negative, proteins take the place of carbohydrates in oxidation. In cases in which the basal metabolic rate was even higher, Bansi found a respiratory quotient as low as 0.700 [1,334]. In these cases, with negative nitrogen balance, fat may constitute 80 to 100% of the material oxidized, with protein forming the remainder. In general, in untreated thyrotoxic patients with BMR of $+30$ to $+50\%$, one may assume that the carbohydrate reserves are exhausted, the body proteins are catabolized to a small degree, and the primary requirements of oxidation are satisfied by fat.

Clinically, these facts are indicated by a reduction of the subcutaneous fat and body protein (especially the protein of muscle), with resulting loss of weight and emaciation. In cases which are not too severe, the loss of weight and the negative nitrogen balance can be successfully counteracted by forced feeding of proteins, carbohydrates, and fats.

The importance of thiamin for the normal reactions of the citric acid cycle has already been discussed (p. 130). In hyperthyroidism, a deficiency of thiamin occurs, deficiency of intermediate products in the cycle follows, and the utilization of oxygen may be reduced.

Proportional to the elevated oxygen utilization, there is an increase in the endogenous formation of water. The amount of exogenous water is determined by the water content of the food and drink and, because of the increased needs for food and drink, is increased in hyperthyroidism. In advanced cases, from clinical observation, the water content of the skin and the muscles seems to be decreased. The loss of protein is accompanied by a loss of water of hydration. The renal excretion of water may be increased. The extrarenal loss of water in the perspiration is increased in hyperthyroidism. There is an increase of the water lost through the lungs as well as the skin amounting, according to DuBois, to 40 grams of water per hour [335]. The patient perspires excessively. Szél [336] and Jores [337] likewise found considerable loss of water by evaporation. In general, the total water balance may at first be returned to normal and not become negative until an advanced stage of the disease.

The metabolism of carbohydrates in hyperthyroidism is altered because of the increased requirements for substrates. The glycogen stores, as already discussed, are depleted. If glucose is administered from the outside, the respiratory quotient goes

up because of the prompt utilization of the glucose. If glucose is given intraperitoneally to hyperthyroid rats after a 48 hour period of fasting, the glucose is utilized to such a degree that less glycogen is synthesized than normally [338]. The relationships between diabetes mellitus and hyperthyroidism probably depend on this phenomenon. The glucose tolerance curve is not characteristic in patients with hyperthyroidism. Following the oral dose of glucose, intestinal absorption is accelerated, and the curve is elevated and prolonged, according to the severity of the disease. The galactose tolerance test following oral administration is also markedly affected. Following intravenous administration, however, the curve is usually normal.

In hypothyroidism there are marked changes in lipid metabolism. In hyperthyroidism, on the other hand, although there is a tendency to a reduction of the lipids (especially the cholesterol), the reduction is only slight. As a rule, only the level of serum cholesterol is reduced. Hurxthal reported that an average BMR of -66% was accompanied by an average serum cholesterol of $82 \text{ mg}\%$, with his lowest value $58 \text{ mg}\%$ [339]. According to Boyd and Connell [308], the phospholipids in 43 patients were reduced to an average value of $125 \text{ mg}\%$, as compared with the normal average of $195 \text{ mg}\%$.

The serum proteins are normal in most cases, or at the lower limit of normal [1]. The albumin fraction may be reduced (e. g., to $2.65 \text{ grams}\%$; normal, $3.74 \text{ grams}\%$). The nitrogen is excreted chiefly as urea, although Kreeh [303] found an increased excretion of amino acids in the urine which was roughly parallel to the increase of the basal metabolic rate.

Creatinuria occurs in starvation, after bodily effort, in hyperthyroidism, and in certain other disorders. The creatine level of the blood is elevated, so that the threshold level of 0.4 to $0.5 \text{ mg}\%$ is exceeded, and creatine appears in the urine. The excess of creatine could be the result either of increased synthesis or disturbed utilization. There is no increased synthesis in hyperthyroidism [309, 340], and it is therefore assumed that there is impairment of utilization of creatine in the creatine phosphate-ATP system. Buell has shown that the creatine phosphate content of the heart of rabbits and cats poisoned with thyroid hormone falls [341]. Wang similarly found that the amount of creatine and creatine phosphate in the muscles of hyperthyroid rabbits is reduced [341]. Various observations have shown that the creatine concentration of the liver, muscles, and heart of patients with hyperthyroidism is reduced. When a loading dose of 1 gram of creatine is given to patients with hyperthyroidism, some 58% is retained within the body (normal, $70-80\%$) [309]. Probably, less creatine is converted to creatine phosphate than in the normal. Many studies have also shown that the oxidative synthesis of adenosine triphosphate (in coupling with the synthesis of creatine phosphate) is reduced in the mitochondria of tissues of hyperthyroid animals (liver, kidneys, diaphragm) (see p. 176). Correspondingly less creatine is converted to creatine phosphate.

The elevated oxidative metabolism in hyperthyroidism also manifests itself in the increased amounts of energy which are produced. This energy is converted into cellular work (physical and chemical) and finally into heat. Charcot found a body temperature of 100° (101.5° in the evening) in one of his hyperthyroid patients [318], and the temperature elevation in this disease has been repeatedly verified. In severe cases, autemortem temperatures as high as 104° to 106° may occur. The usual accompaniments of fever are not present [323]. The increased production of heat in hyperthyroidism is largely compensated for by an increased output of heat by the mechanisms of heat regulation. It is only in severe cases that the rise of temperature is pronounced.

The amount of cellular work can be measured by the muscular work. The relationship between oxidative metabolism and muscular work has been measured for many years. The patient with hyperthyroidism requires more energy than normal for a given amount of work. The impaired muscular strength was observed by Müller in 1867 [323]. In 14 cases studied by Boothby and his coworkers [342], the energy required to walk was twice that required by the normal person. The efficiency of muscular work, in other words, is reduced. Following effort, the blood level of lactic acid returns only slowly to normal, despite the increased utilization of oxidative substrates.

Bassi felt that the muscle tremors of hyperthyroidism, so well described by Komaroff [343], are not related to the poor efficiency of the muscles [1]. The tremors may require additional energy and be responsible for a corresponding amount of heat. If one extrapolates the resting energy utilization of the increased muscular tonus, an increased basal metabolism is still present [344, 345]. Finally, there are hyperthyroid patients with increased basal metabolic rates in whom there is no increase in muscle tonus [344].

The efficiency of the cardiovascular system is affected by the impaired cellular energy state. In vivo and in vitro studies of cardiac muscle of animals show elevated oxygen utilization by cardiac muscle. The cardiac output then depends on the characteristic tachycardia. The pathogenesis of the tachycardia is not known. The rate at rest varies from 80 to 130 per minute in different patients [346]. The tachycardia is independent of the nervous system, is not related to respiration, and is present even during sleep. The stroke volume is normal [347], so that the minute volume depends on the heart rate. The normal minute volume is 3 to 4 liters per square meter of body surface; that of the thyrotoxic heart is 5 to 11 liters [349]. A 35% increase of basal metabolism corresponds on the average to a 45% increase of the minute volume [348]. The increase in the cardiac output is a reaction to the high oxygen requirement of the body tissues. When the work load increases further, the heart beats even faster, and the limit of cardiac reserve, which is markedly reduced, may be reached rapidly.

The peripheral circulation is characterized by an increased blood flow. The circulation time is decreased [352]. The peripheral resistance is lowered, except in the brain [353], lungs, and liver [354]. The blood volume is normal or increased [350, 351]. The blood is distributed chiefly to those tissues whose metabolism is especially increased and to the thyroid glands. There is increased blood flow to the muscles, skin, kidneys, lungs, and thyroid. The cause for the increased blood flow is not only the increased oxygen requirement but also the increased requirement for substrate and the increased requirements of heat regulation.

A normal electrolyte milieu is a prerequisite for normal function of the heart and skeletal muscle. The deficit of energy in hyperthyroidism is also evidenced by a disturbance of this function of the cells. The extracellular potassium is elevated (it is reduced in myxedema) and the intracellular potassium is reduced. Together with the latent deficiency of high-energy phosphate, this intracellular deficiency of potassium ions is a further basis for the inefficient performance of cardiac and other muscles.

As to hypothyroidism, the question presents itself in hyperthyroidism as to whether the oxygen transport function of the circulation is adequate. The arterial oxygen saturation is normal [354], the function of the lungs is normal, and anemia is not present except in advanced stages of the disease. The arteriovenous difference of 5.8 volumes% is also normal [354]. Available data of the oxygen supply to the splanchnic area (liver) and the brain show that the supply of oxygen is adequate. There are

no electroencephalographic signs of hypoxia. The heart muscle also has a good oxygen supply and angina pectoris and myocardial infarction are rare [354a]. Hypoxic electrocardiographic changes do not usually occur. Thus, when the oxygen content of the air is normal, no signs of oxygen deficiency appear; i. e., at normal barometric pressure, the oxygen supply of the body is adequate. The hyperthyroid individual is, however, very sensitive to external oxygen deficiency, as at high altitudes and in carbon monoxide poisoning.

The functions of the liver are usually impaired. The hippuric acid and galactose tests are positive; both these tests depend on enzyme chemistry (p. 165), and are therefore of special pathogenetic interest. The disturbed production of energy in hyperthyroidism is seen here in certain pathological reactions. Thus, the synthesis of prothrombin is impaired, for this is a protein transformation which requires energy and, under conditions of uncoupling, can no longer proceed optimally. The functions of the liver are further impaired by the glycogen deficiency and the preexisting protein deficiency due to the constant loss of nitrogen.

The tissues and the circulation adapt themselves to the increased stress but become progressively less and less efficient. The peripheral and autonomic nervous systems have a fundamental effect on the development of the syndrome. The sympathetic nervous system is not necessarily overstimulated, and does not always, therefore, influence the clinical picture. The well-known hypersensitivity of hyperthyroid patients to epinephrine, the cause for which is not known, is probably also related to changes in the irritability of the nervous system [355].

As the disease progresses, there are progressive increases in the basal metabolism, the heat formation, and the utilization of substrate. Vitamin deficiencies develop. Secondary complications involving individual tissues ultimately occur. Extreme hyperthyroidism is known as thyrotoxic crisis: the temperature is markedly elevated, the BMR elevation is severe, and there are acute manifestations of failure of the heart, liver, intestinal tract, nervous system, adrenal glands, and other organs.

Clinical Hyperthyroidism

Graves' disease or Basedow's disease (hyperthyroidism) occurs among all races, in women more often than in men, and in all ages (especially in the third and fourth decades of life). There is no hereditary tendency, and no constitutional predisposition has been documented. The amount of iodine in the diet, however, may be important in the development of the disorder.

In most cases, the etiology is not known. Precipitating factors include psychic trauma, endocrine changes (such as pregnancy, menopause, puberty), and chronic infections. In addition, hyperthyroidism may occur in association with carbon monoxide poisoning, heavy metal poisoning, and iodine intoxication.

The disorder is gradual in its development, usually taking weeks to months to become established. In some cases it may not develop for over a year; in others, however, it may become full-blown in a matter of days. The initial symptoms vary, and often cannot even be recalled by the patient.

The classical syndrome includes tachycardia, enlargement of the thyroid (goiter), and exophthalmos — the so-called "Merseburger triad" described by Karl von Basedow in 1840 [285].

Tachycardia is rarely absent. At rest, the heart rate is 80 to 130 per minute, and this rises to 160 or more following effort. Spang and Korh [346t] found a parallel

between basal metabolism and heart rate up to 120 beats minute, but not above this rate. The tachycardia persists during sleep. In later stages of the disease, arrhythmias occur, in some 15% of all patients [346]. Auricular fibrillation may be paroxysmal or constant. The heart, which is at first normal, may become dilated. On auscultation, the heart sounds are loud, the second pulmonic sound is accentuated, and a systolic murmur may be present. There are no characteristic changes in the electrocardiogram. The systolic blood pressure is normal or elevated, and the diastolic blood pressure falls. The patients complain of pressure over the chest and palpitation. All these thyrotoxic heart changes are reversible unless actual anatomic damage to the heart supervenes.

The typical goiter is a diffuse, symmetrical enlargement of the thyroid gland. There is marked enlargement without pressure symptoms. The gland is boggy in texture, and a thrill and an audible murmur may be present. The thyroid is extremely vascular and its blood flow is increased (p. 166). In contrast to this typical picture, how-

ever, there is the toxic adenoma, in which the syndrome of hyperthyroidism is accompanied by local adenomas which are circumscribed and hard and may produce local pressure symptoms.

Exophthalmos is bilateral and usually asymmetrical, but may sometimes be unilateral. At first, the eyeball can be pushed back to its normal position, but later the exophthalmos becomes fixed within an edematous retrobulbar space (Fig. 51). Other eye signs include difficulty in convergence (Möbins), lid lag (Graefe), diminished blinking (Stellwag), glint (Rosenbach), and lid edema (Chvostek) [1]. If the typical eye signs are present, the diagnosis may be made at a glance by the staring, frightened expression of the patient. The patient complains of spots before the eyes, photophobia, and easy tearing. Conjunctivitis, corneal ulcerations, and blepharitis may complicate the picture. The degree of exophthalmos is not proportionate to the severity of the hyperthyroidism. If the exophthalmos becomes progressive, with conjunctival and corneal complications and danger of blindness, the term "malignant exophthalmos" is used.

The pathogenesis of the exophthalmos is not known. It is generally assumed to be due to a pituitary effect of some sort, as evidenced by the improvement following irradiation of the pituitary and the production of experimental exophthalmos by means of thyrotropic hormone [356]. Thyrotropic hormone has not, however, been sufficiently purified and contaminants may have been responsible for the latter results.

In addition to this characteristic triad, there are other signs in the disease. Among these are the fine tremor of the tongue and the extremities and the increased irritability of the patient. The irritability shows up in the cardiovascular system, in the tendon reflexes, and in the intestinal motility (diarrhea). Insomnia is common, emotional stability is poor, and depression, euphoria, and even psychoses may occur.



Fig. 51. Typical goiter and exophthalmos in a 23 year old patient with hyperthyroidism. BMR = + 67%. (From the Medical Polyclinic of the University of Munich.)

The skin is soft, warm, and moist. Chloasma-like pigmentation may be present. There is loss of the fine hair. Perspiration is increased. There is often slight elevation of body temperature. The patients are sensitive toward heat and prefer cool rooms and cool seasons of the year. Weakness and easy fatigability are present both at rest and following effort.

The blood picture is unchanged. Occasionally there is a tendency to hypoproteinemia and bleeding. Gastric hypoacidity is present and, later, gastric anacidity. Osteoporosis may occur if emaciation is present.

Signs and symptoms vary from patient to patient. Asthenia, cardiac signs, eye signs, or intestinal signs may predominate. Thyrotoxic crisis may develop suddenly, with vomiting, diarrhea, asthenia, tachycardia, restlessness, high fever (40–41° C.), dehydration, coma, and death. When untreated, hyperthyroidism usually goes on for many years with long remissions, usually in spring. In each phase, symptomatology gradually becomes maximal, and then gradually subsides. Not infrequently, the picture is converted to myxedema, perhaps because of exhaustion of the thyroid gland.

The diagnosis is simple if the typical picture is present. Important points are the elevated basal metabolic rate, the elevated protein-bound iodine, the characteristic radio-iodine test (reduced excretion in the urine, increased uptake by the gland, accelerated hormone phase), the reduced serum cholesterol, and the reduced excretion of creatine in the urine. The disease must be differentiated from other causes of elevation of the basal metabolism (Table 11). The most important diagnostic tool is the determination of the blood iodine, together with the use of a tracer dose of radioactive iodine. Only pregnancy and age affect both the blood iodine and the basal metabolism. In general, hyperthyroidism is not present if the iodine circulation is not increased and the BMR is not elevated. However, cases of "masked hyperthyroidism" occur, in which the iodine circulation is increased but the BMR does not show corresponding elevation [259]. However, in such cases, the question arises as to whether the basal metabolism is measured under optimal conditions. A latent deficiency of vitamin B₁, which can occur in hyperthyroidism, can prevent the maximal increase of basal metabolism which would correspond to the accelerated iodine circulation. This maximal increase first shows up when the deficiency of thiamin is corrected.

Diagnostic difficulties may occur if hyperthyroidism is associated with obesity or if severe muscular weakness and tremors are present. Hyperthyroidism with obesity is fundamentally the same as hyperthyroidism in normal or thin persons, except that the appetite exceeds the dietary requirements so that the patient does not burn his own body tissues. Tachycardia, generalized nervousness, severe diarrhea, changes in the skin and hair, and slight enlargement of the thyroid gland are all present and suggest the correct diagnosis. Determination of the basal metabolic rate and testing with radioactive iodine are diagnostic.

The diagnosis of hyperthyroidism in children, which is characterized especially by marked longitudinal growth of the bones, is made difficult by the difficulties of determining the BMR in children. In addition, creatinuria is physiological in children. In these cases, the diagnosis must be made by means of the blood iodine determination.

Tuberculosis may simulate typical Graves' disease, and the same is true of other chronic subfebrile illnesses. In such cases, the symptoms related to the total metabolism are of less importance, since tachycardia and increased basal metabolism also occur with fever. More important are the signs and symptoms due to the thyroid glands: enlargement of the thyroid (goiter), exophthalmos, and accelerated iodine circulation. If the circulation of iodine is normal, hyperthyroidism is not present. A

normal BMR also is evidence against hyperthyroidism, with the exception of the rare cases of "masked" hyperthyroidism. In disorders of the sympathetic nervous system, the blood iodine is normal although the BMR is high. Yet, this increased BMR is not a true increase of oxidative basal metabolism, but denotes increased function as a result of increased reflex muscle tonus. When cardiac function is increased, signs of increased circulation and systolic hypertension are present [374].

The management of hyperthyroidism is quite symptomatic, since the fundamental cause is not known. The chief method of treatment is directed at the circulation of iodine. Theoretically, this can be influenced at the peripheral tissue cell, within the thyroid gland, or by way of the pituitary-thyroid axis. The inhibition of the peripheral action of the hormones by hormone antagonists is primarily of theoretical interest (p. 176). The modification of iodine circulation within the thyroid gland is accomplished by surgical, physical, or biochemical measures. The simultaneous control of the activity of the pituitary has been shown to be a useful method. These measures usually cause improvement in the clinical picture and thus improvement of the energy balance of the body.

The purpose of surgical and physical measures is to reduce the amount of active thyroid tissue. Surgically, most of the gland is removed, with a small amount left behind to allow normal thyroid function. Physical treatment consists of the administration of radioactive iodide (I^{131} , 100 to 250 microcuries per gram of gland) which is stored within the thyroid in the same way as the natural isotope and, by means of its radiation, reduces the activity of the gland [379, 380]. The principles are the same as those formerly used for the use of x-rays in hyperthyroidism. It is necessary that the thyroid have a sufficiently high avidity for iodine, as determined by *in vivo* counting after a tracer dose. The previous administration of iodine for therapeutic or diagnostic purposes (Lugol's solution, diiodotyrosine, gall bladder dye, etc.) or of anti-thyroid drugs may so affect the thyroid that it takes up little iodine for several months. In such cases, radioactive iodine cannot be successfully administered, for it is not taken up by the gland. The advantage of treatment with I^{131} is the ease of its administration and the localized nature of the resulting irradiation. Disadvantages include the difficulty of determining proper dosage, for the sensitivity of a particular gland to radiation cannot be predetermined exactly. The weight of the gland is estimated, as well as the degree to which it stores iodine, and the duration of such storage (tracer testing). In typical cases of diffuse hyperplasia with hyperthyroidism, there is rapid improvement after treatment [259]. Toxic adenomas are more resistant [259]. Carcinogenic action is still a theoretical possibility, so that only patients above the age of 45 are recommended for such therapy [358]. Some authors, however, treat patients as young as 25 to 30 years of age [345]. Pregnancy is a contraindication to the use of I^{131} , as is also the presence of severe compression symptoms. Sometimes, especially when the dose is too high, the irradiation causes acute tissue damage and therefore outpouring of hormone iodine and thyroglobulin into the bloodstream, with transient aggravation of the symptoms of thyrotoxicosis.

The circulation of iodine can be inhibited within the thyroid gland at two locations: at the stage of concentration and fixation of iodine, or at the stages of iodination or condensation (i. e., the actual synthesis of thyroid hormone) (Fig. 50). The concentration mechanism can be inhibited by iodide analogs such as thiocyanate and perchlorate. The second stage can be inhibited by various antithyroid, goitrogenic substances, especially the thioamido derivatives such as thiouracil [292]. Finally, the total function of the thyroid can be inhibited by large amounts of inorganic iodine, first introduced by Plummer [359].

The action of the iodide analogs depends on their relationship to the halogens. They compete successfully to replace the iodide ion from its site of concentration or fixation within the thyroid cells. Following the administration of thiocyanate or perchlorate, for example, the total inorganic iodine fixed within the thyroid but not yet organically bound is suddenly displaced and passes into the bloodstream and out in the urine. There results a "discharge" of iodide from the thyroid gland, and therefore cessation of hormone synthesis. The reaction of the thyroid is the same as to an exogenous deficiency of iodine (Table 49, Fig. 50). If the ingestion of iodine is resumed at the same time, transient hypothyroidism may follow [256]. Because of its side reactions, potassium thiocyanate cannot be used therapeutically. The perchlorate ion is ten times as active, and potassium perchlorate has been used in doses of 200 to 800 milligrams per day [360]. It has no side effects on the hematopoietic system, but has little goitrogenic effect on the disease process by itself. It is also used prior to operation especially when there is marked sensitivity to iodine [382, 383].

The functional group of the thioamido compounds is a free or bound SH group, which is responsible for its reducing properties and therefore for its reaction with iodine. Pitt Rivers [361] showed that this property parallels the biological action of the compounds in the thyroid. The thioamido derivatives inhibit the iodination of tyrosine and the condensation reaction. The exact mechanism of the reaction is not known. Thus, the thioamido compounds inhibit the synthesis and therefore the secretion of thyroid hormone (Figure 50). The ability to concentrate iodine remains. Since synthesis is blocked and the accumulated inorganic iodide is therefore not utilized by the thyroid gland, and since the iodide continues to be produced by degradation of the hormone in the peripheral tissues, there results an increase of iodide in the blood and the extracellular space, and an excretion of large amounts of inorganic iodine in the urine. The thioamido compounds have been successfully used in the treatment of hyperthyroidism since their discovery by Astwood in 1943. Their disadvantage is the production of a goiter: The inhibition of hormone synthesis results in a peripheral deficiency of hormone, which in turn leads to an overproduction of thyrotropic hormone which stimulates hypertrophy of the thyroid gland. Although the gland is empty of hormone, it develops an increased blood supply and an increased surface area (goiter). For this reason, such a gland is frowned upon by the surgeons; i. e., the use of the thiourea drugs preoperatively is contraindicated. Propylthiouracil is one of the best of the original drugs of this group. In 1949, Stanley and Astwood found new antithyroid drugs among the mercapto-imidazol compounds [362], among which 1-methyl-2-mercapto-imidazol has only slight goitrogenic action but is 100 times as effective as thiouracil as measured by the inhibition of the uptake of I^{131} .

The dosage of the thioamido compounds depends on the state of nutrition and on the individual sensitivity of the patient. Treatment begins with 40 to 80 mg (at most, 100 mg) of 1-methyl-2-mercapto-imidazol or 100 to 400 mg of propylthiouracil, and continues until the clinical findings become normal. Finally, a small maintenance dose is determined which is used for months or longer (10-20 mg of the imidazol derivative, or 50-100 mg of propylthiouracil).

The clinical danger of this group of compounds lies in their side reactions, which include disturbances of the skin, the mucous membranes, and the bone marrow. Agranulocytosis may occur and may result in death. In 1947, Himsworth and Trotter [363] collected 928 cases so treated, with 207 patients showing agranulocytosis, and with death in one-fourth of these. The same types of side reaction occur with the imidazol preparations [364]. The treatment is considered harmless in pregnancy, according to Astwood [365].

The pathogenesis of the side reactions is probably related to the fact that the thiouracils are structural analogs of uracil, which is a component of the nucleotides and ribonucleic acid. It can be shown that thiouracil can be built enzymatically into mononucleotides [264], so that the synthesis of nucleonides with a false base is possible. In this connection it is of interest that the bone marrow, with its high rate of cell turnover, is the tissue most affected by the side reactions. Roche and coworkers [267] showed further that thiouracil affects not only the synthesis of thyroid hormone, but also the synthesis of globulin within the thyroid gland, resulting in a qualitatively different protein. The close relationship between the nucleic acids and the synthesis of proteins makes the fact that uracil derivatives can also influence protein synthesis readily understandable. The biochemical mechanism of the side reactions of the imidazol drugs is not understood.

It was Plummer who introduced the use of large doses of iodine (6 or more mg of iodide) in the treatment of hyperthyroidism. Such treatment causes cessation of secretion of thyroid hormone and the storage of the hormone within the gland. The amount of colloid increases, the gland becomes smaller and firmer to the touch, the hyperactive gland becomes less active, the rate of blood flow through the gland is reduced, and local signs disappear. Wolff and Chaikoff [260] showed inhibition of hormone synthesis following the administration of iodide in experimental animals, and such inhibition must also occur in the human being. Iodine has long been used preoperatively in hyperthyroid patients, the usual maximal dose being some 55 milligrams of inorganic iodine (as Lugol's solution) in 10 days. Besides its preoperative use, iodine is valuable in the treatment of thyrotoxic coma. In addition to iodine itself, diiodotyrosine is of value here [368], and corticosteroids are usually necessary in addition. Diiodotyrosine is broken down within the body to tyrosine and inorganic iodine, 100 mg of diiodotyrosine giving 59 mg of iodine. Treatment consists of the daily administration of 100 to 800 mg of diiodotyrosine, orally or intravenously, for about 2 weeks, until a satisfactory result is obtained. At this point, treatment is gradually shifted to methyl-mercapto-imidazol, at first using both preparations together, and then gradually eliminating the diiodotyrosine. Some authors give both preparations together from the very start [1].

The choice of treatment must be individualized. Toxic adenomata and postoperative thyrotoxicosis respond less well to the use of the thiouracil derivatives and radioiodine than diffuse hyperplasia of the gland. When hyperthyroidism is mild, the antithyroid drugs are the method of choice in many cases. Initial treatment may employ an imidazol derivative, with change to propyl thiouracil when the patient becomes resistant, or the reverse procedure may be used. In moderately severe cases, the physician must choose between the antithyroid drugs and I^{131} . Many workers combine the use of I^{131} with moderate doses of propyl thiouracil (100 to 200 mg/day) [369]. This combination is especially useful in the age group of 30 to 45. Surgical treatment remains necessary for cases in which the thyroid gland is very large and resistant to thioamido and I^{131} therapy, as well as for toxic adenomata, adenomata with pressure symptoms, and carcinoma.

General measures include proper diet to satisfy the metabolic requirements, and sedation for the nervous symptoms. Rest in bed is important. The diet must be high in calories (up to 5,000 calories per day) and adequate in its content of nitrogen, vitamins, and trace elements.

The psychic manifestations vary from one patient to the next, and must be treated individually.

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CHAPTER III.

Carbohydrate Metabolism The Biochemistry of Diabetes

By Joachim Kühnau and Claus von Holt

Introduction

The development and maintenance of life on the earth require two prerequisites: the formation of specific physico-chemical substances in which the phenomenon of life can manifest itself, and the existence of active chemical mechanisms around these substances which, by a constant production of energy, maintain the specific nature and particularly the high degree of orderliness of the living substance. These mechanisms also guard against sickness and death, events which are accompanied by a reduction of the high degree of orderliness. Of the three principal foodstuffs, which are also the three chief components of body tissues, the proteins are of importance as the substances which carry the expressions of life (the first of the prerequisites), and the fats as the agents which provide energy (the second prerequisite). The carbohydrates, however, are of greatest importance in *both* the structural and energetical senses, and therefore exert especially broad and fundamental influences on the processes of life. Their structural functions have long been known among plants, but it is only in recent years that complex carbohydrates bound to proteins have been shown to have a profound importance in the synthesis of animal tissues, especially the cell membranes and the supporting and connective tissues, as well as the intercellular ground and cement substances. The carbohydrates also appear to be involved in the specific reactions of the animal body as a result of the part they play in the synthesis of hormones, antibodies, blood group substances, etc.

Just as important and even better known is the role of the carbohydrates in fulfilling the second of the prerequisites mentioned above; that is, in the supply of energy. They are more important than the fats in this respect because the function of the fats as a source of energy is dependent on the simultaneous presence of certain combustible degradation products of glucose or amino acids. In addition, the fats cannot be utilized at all by certain tissues, such as the nervous system. As a result, the carbohydrates turn out to be the most important substances in the supply of energy for the animal body, and their constant availability in an easily utilizable form is essential for life. This form is D-glucose, which McLeod has therefore called the "fuel of life."

There are two mechanisms which ensure that glucose is always available in adequate amounts for use by the cells: (1) the intake of carbohydrates, which form the largest portion of our daily diet and which break down to glucose in the process of

digestion; and (2) gluconeogenesis, a process by which glucose is formed from body protein or fat during times of relatively or absolutely increased demand for glucose.

Under normal conditions, both of these processes, which are closely related to each other, can attain their goals only if the metabolic processes as a result of which the energy contained in the glucose molecule is set free, function normally; that is (p. 202), if the utilization of glucose within the body is not disturbed. The problem of the pathogenesis of diabetes becomes relevant at this point. Minkowski believed that the fundamental abnormality in diabetic metabolism is impaired utilization of glucose, and this view has been verified by isotope studies [1]. The result is, at the very least, danger to the one fundamental process of maintenance of life, the production of energy. This danger stimulates the diabetic body to compensatory measures which take place at the expense of synthesis of cellular and tissue protein and thus also affect the first fundamental prerequisite, the synthesis of the living structures of the cells, which are protein in nature. In addition, radioactive studies of the intermediary metabolism of individual chemical substances have shown that the stage in metabolism which precedes the degradation of glucose – namely, the uptake of glucose into the cells – is abnormal in diabetes. These studies have further shown that the various pathways of metabolism and catabolism of glucose are partly altered and partly completely blocked, so that widespread or even complete blocking of the oxidation of glucose results. These blocks may occur at various points in the chain of glucose catabolism and can vary in their intensity. They may also be due to different etiologic mechanisms. As a result, it would be improper and false to think of the disturbance of metabolism in diabetes as a single independent self-contained abnormality and to say that, under all circumstances, the defect is always due to a deficiency of insulin. It has been shown, for example, that disturbances in the utilization of glucose similar to those disturbances which occur in diabetes may also occur in the presence of normal body insulin; and, on the other hand, it has also been shown that carbohydrates may be normally utilized even when insulin is completely absent (p. 227).

The complex nature of the metabolic disturbance in diabetes is due chiefly to the fact that the diabetic individual counteracts the threat to his energy supply by means of a number of adaptive reactions. The threat to the supply of energy is due to diminished oxidative degradation of glucose.

The compensatory mechanisms have as their common denominator an increased rate of breakdown of glucose, accomplished by increasing the amount of glucose present within the body, which thus leads to increased rate of catabolism. The disturbance in glucose breakdown varies in degree from patient to patient, but is always greater than its counterpart, gluconeogenesis. Soskin and Levine showed that the resulting increase in glucose available for metabolic processes is the means by which the imbalance between energy available and energy required may be corrected. All enzymic processes within body cells conform to the law of mass action, which states that, all things being otherwise equal, the rate of passage of a given substance through a given metabolic process depends on the initial amount of the substance. If there is reduced activity of one or more of the enzymes involved in the reactions of glucose breakdown, glucose may be catabolized in amounts insufficient to supply the required energy. In such a case it is possible, by supplying glucose to excess, to increase its metabolism to normal (Fig. 52) [2, 3]. From this point of view, diabetic hyperglycemia has a two-fold importance: (1) it is a pathological result of disturbed metabolism of glucose; and (2) it is also an important physiological adaptive reaction which permits the diabetic organism to maintain the energy supply which is other-

also threatened by the disturbed oxidation of glucose. For this to be accomplished, it is necessary to have, especially between meals, higher blood levels of glucose than could be produced by the accumulation of glucose following stoppage of glucose metabolism alone.

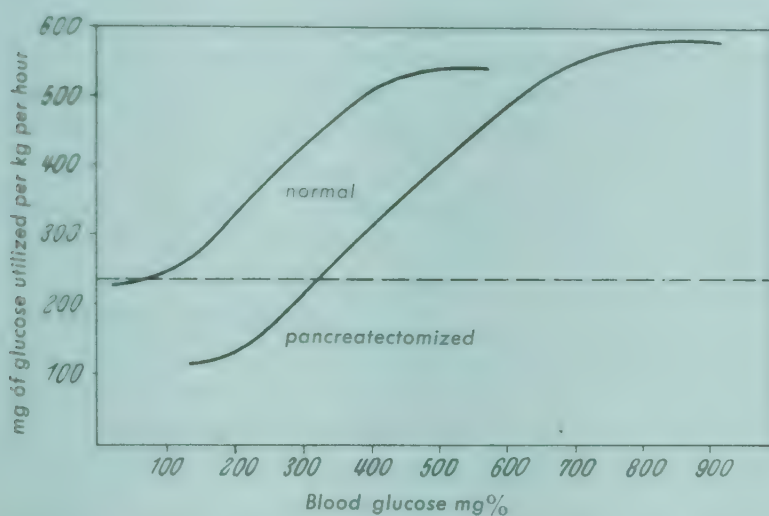


Fig. 52. The relationship between the utilization of glucose and the level of blood glucose. From Soskin and Levine. The dotted line represents the normal utilization of glucose.

The additional, often decisive mechanism which supplies glucose independently of the diet is gluconeogenesis. Still another mechanism is increased glycogenolysis with the formation of glucose in the liver. Both these mechanisms are of importance in the supply of energy in the diabetic body (p. 204). They are under the control of the adrenal cortex and are accompanied by an increased production of corticosteroids (p. 232). It is only with the help of these processes that the tissues of the untreated diabetic can maintain their normal functions. The importance of the adrenal cortex in the development of diabetes is also shown by the disappearance of hyperglycemia and glycosuria in pancreatic diabetic animals which undergo adrenalectomy [4] (p. 229). In diabetes, the disturbed breakdown of glucose (Minkowski) and gluconeogenesis (the "overproduction" theory of von Noorden) are not mutually exclusive, as formerly believed, but go on side by side. The augmentation of the energy content of the diabetic body by the adrenal cortex must be paid for dearly in the absence of insulin, which returns the oxidation of glucose to normal and thus corrects the stimuli which cause hyperglycemia and gluconeogenesis. Longstanding hyperfunction of the adrenal cortex may occur in florid, untreated diabetes and in diabetes in older persons, and may have deleterious effects on the human body (glomerulosclerosis and retinopathy of Kimmelstiel-Wilson). Especially in older patients with diabetes and Kimmelstiel-Wilson syndrome, certain endocrine changes may develop, with increased activity of the pituitary-adrenal cortex axis. The resulting gluconeogenesis may become the prominent finding in the pathogenesis of the diabetes, in contrast to the usual disturbance of glucose breakdown found in other types of diabetes, and increasing resistance to insulin follows. Bertram calls this type of diabetes "counter-regulatory diabetes," and states that it occurs chiefly in elderly individuals of pyknic body build and a tendency to hypertension. He contrasts it to "insulin-deficiency diabetes," which occurs in younger, thinner, hypotensive individuals, in whom the chief abnormality is dis-

turbed utilization of glucose. In rare cases, hyperplasia or adenomas of the adrenal cortex can lead to another, atypical form of insulin-resistant diabetes known as "steroid diabetes" (p. 231).

The recognition of the presence of a complex disturbance of carbohydrate breakdown in diabetes comes from the observation that this disturbance runs parallel to the disturbances of metabolism which occur in prolonged starvation. In starvation, the oxidation of carbohydrates is also reduced to a minimum determined by the amount of gluconeogenesis, and many metabolic abnormalities which occur in diabetes (p. 218) are also found in starvation. These similarities must be viewed merely as the results of diminished carbohydrate metabolism and give no clue to the different causes in the two sets of circumstances. However, prolonged starvation may be followed by other changes in the enzymes of carbohydrate metabolism, which may lead to further similarities to diabetes and, if carbohydrate is then suddenly administered, to a true "hunger diabetes" (Hofmeister) [5]. The development of this type of diabetes, described in 1859 by Claude Bernard and known to the older clinicians as "vagantenglucosuria," is due to the fact that some or all of the enzymes of carbohydrate catabolism are present and active in their entirety only when they are needed, even in the non-diabetic individual – i. e., when carbohydrates are present for utilization. They decrease and are no longer formed when prolonged lack of carbohydrates eliminates the need for them. When carbohydrate is then suddenly given to such starved individuals, the lack of these enzymes prevents the body from utilizing the carbohydrate and it is excreted as glucose.

Apparently, many of the enzymes concerned in the metabolism of carbohydrates belong to this group of "adaptive" or "induced" enzymes, which are synthesized only when needed and only by the specific substrate on which they are to act. These concepts have been verified by recent isotope experiments in animals: both in prolonged starvation [6, 7] and in rats fed on a carbohydrate-free high-fat diet [8, 9] there is increasing impairment of phosphorylation of glucose, synthesis of glycogen, and breakdown of glucose to carbon dioxide in the liver and the muscles. Both starvation and a low carbohydrate (high-fat) diet impair carbohydrate tolerance [10, 11]. This fact is of practical importance and helps in the planning of diabetic diets. The relationship of the diabetiform aberrations caused by prolonged withholding of carbohydrates to true diabetes is further emphasized by the fact that in hunger diabetes too the disturbed breakdown of glucose is accompanied by gluconeogenesis resulting from increased pituitary-adrenal cortical activity.

From these biochemical considerations, it can be seen that the metabolic events of diabetes must be studied under two large headings; namely, disturbed breakdown of glucose and disturbed production of glucose. Each of these complex events must further be broken down into two parts, study of the individual processes which make up each disturbance, and investigation of the governing (i. e., hormonal) mechanisms involved.

General Features of Carbohydrate Metabolism in Diabetes

The multiple disturbances of carbohydrate metabolism which are the fundamental abnormality in diabetes can be understood only if one understands the individual reactions which occur in this metabolism. Since each reaction is catalyzed by specific enzymes, a knowledge of basic enzymology is essential. Two particular laws are of

great importance: the *law of mass action*, and the *reversibility of enzymic reactions*. If one considers these facts in order, it is immediately clear that within the chain of catabolic reactions, which represent a constant fluid equilibrium with steady inflow and outflow of materials, an impairment of the efficiency of each component reaction can prevent the functioning of both the preceding and the subsequent components of the chain - the preceding by accumulation of the reacting substances, and the subsequent by absence of materials. Under special circumstances, when the reaction rate is markedly reduced, the reaction may proceed in the reverse direction - i. e., to synthesis. This event at first affects the antecedent reactions, but later also affects the subsequent components, especially when some end products of the reaction chain are interposed as a result of unaltered side reactions. In this way, an enzymic block which affects the late steps of the catabolic process and which is not involved in the breakdown of glucose itself can result in the accumulation of glucose with **accompanying hyperglycemia and glycosuria**.

In general, a normal transfer of glucose through the catabolic chain is linked to a definite, constant ("stationary") concentration of the individual intermediary products of the catabolic process. Any aberration of metabolism of the type seen in diabetes must be manifested by changes in the concentrations of these intermediary substances. These relationships can take on an extremely complicated character if two or more enzymes are concerned in the metabolism of a single intermediary substance, so that "branching" of metabolism may occur as, for example, at the stage of glucose-6-phosphate or pyruvic acid. If the substrate affinities of the individual enzymes of the branch reaction which determine the rate of transfer of the intermediary products differ, a change in the concentration of the mother substrate may have different effects on the transfer in the individual branch reactions. Thus, the catabolic reaction may be displaced from the normal pathway towards a side reaction, so that the process may not only be quantitatively diminished but may be qualitatively altered (i. e., shifted into other pathways) [13]. Such events play an important role in diabetes.

If, as occurs in diabetes, it is primarily the oxidative reactions which are impaired - those which are catalyzed by dehydrogenases - the result is changes both of the "stationary" concentrations of the intermediary products and of the degree of hydration of the coenzymes responsible for transferring the hydrogen of the substrate. Under normal conditions, these coenzymes, especially the pyridine nucleotides which transfer hydrogen directly from oxidizable substrate, are present chiefly in reduced (hydrogenated) form. If, however, the oxidative processes are blocked, there is no hydrogen available for hydrogenation of the pyridine nucleotides, so that these are present chiefly in their oxidized form. Hence, the ratio of oxidized to total diphosphopyridine nucleotides (DPN) is increased in diabetes [14]. Similar changes occur in starvation, which resembles diabetes in that less carbohydrate undergoes oxidative breakdown than normally. Since the redox potential of the tissues is largely dependent on the ratio of oxidized to reduced DPN or TPN ($\frac{DPN^+}{DPNH}$, $\frac{TPN^+}{TPNH}$), it follows that in diabetes the redox potential of carbohydrate-utilizing tissues is shifted in a positive direction as compared to the normal. This shift was observed as long ago as 1931 [15].

In this connection, TPNH has a special significance as the particular substance which supplies the hydrogen for the synthetic steps in intermediary metabolism, while DPNH primarily supplies energy [666, 667]. The marked diminution of direct oxidative breakdown of glucose (Dickens-Horecker cycle), which is the chief source

of hydrogenated triphosphopyridine (Fig. 55) is one of the causes for the change of the redox potential and is thus responsible for the decline of synthetic processes in diabetes. The proportion of reduced pyridine dinucleotides determines whether the metabolism proceeds in an anabolic direction (increase of TPNH) or not. The amount of reduced pyridine dinucleotides is governed by the transhydrogenases, enzymes which transfer hydrogen from a reduced to an oxidized pyridine dinucleotide. The possibility is not excluded that part of the metabolic defect in diabetes is the result of disturbances in the transhydrogenase system, as evidenced for example by the antidiabetic action of estrogens, which can function as the coenzyme of transhydrogenases [668, 669].

The decrease of reduced coenzymes in diabetes is one of the causes of the *diminished synthesis of fat* which is present in this disease, for lipogenesis is a reductive process which depends directly on the concentration of TPNH (reduced pyridine nucleotide). A certain minimum concentration of this substance is necessary for the enzymes ketothiolase and ketoreductase to allow synthesis [16, 17]. The increase of the ratios

$\frac{DPN^+}{DPNH}$, $\frac{TPN^+}{TPNH}$ is also a basic cause of the *loss of nitrogen* in the urine and the negative

nitrogen balance which are characteristic of diabetes. This fact follows from the fact that the formation of urea is a function of the concentration of NH_3 , and the production of NH_3 in the liver by glutamic acid oxidation (catalyzed by glutamic acid

dehydrogenase) is in direct proportion to the ratio $\frac{DPN^+}{DPNH}$, $\frac{TPN^+}{TPNH}$ [18].

The change of this ratio is also implicated in another metabolic disturbance found in diabetes, the *diminution of reduced glutathione* without any change in the total glutathione. The hydrogenated (SH-) form of glutathione is decreased as a result of the decrease of the reduced pyridine nucleotides, which can convert oxidized glutathione to reduced glutathione in the presence of specific enzymes [19]. The diminution of SH-glutathione is of great pathophysiologic importance in diabetes, for this substance, as the coenzyme of triose-phosphate dehydrogenase (p. 201), is involved in a key reaction of the oxidative breakdown of glucose. In addition, SH-glutathione has the function of protecting the numerous mercapto-enzymes (p. 201) which take part in carbohydrate metabolism against oxidation and resulting inactivation. This function is probably involved in the hypoglycemic effect of glutathione and other SH-compounds [20]. The various disturbances of sulfur metabolism seen in diabetes can be accounted for on the basis of the physiological functions of the sulfhydryl compounds in carbohydrate metabolism (glutathione, coenzyme A, reduced lipoic acid, mercapto-enzymes).

The tissue content of *high-energy phosphate compounds*, the basic form of chemical energy in metabolism, is markedly diminished in diabetes and in starvation [21]. This is especially true of adenosine triphosphate (ATP). The diminution of high-energy phosphate is the direct result of the diminished degree of hydrogenation of the pyridine nucleotides. The transfer of available hydrogen (which is markedly limited in diabetes) from the pyridine nucleotides to the cytochrome system and then to the oxygen of air, produces on the average 3 high-energy bonds (ATP) per atom of oxygen. If the necessary hydrogen is lacking, the ATP necessary for energy can be produced only in inadequate amounts by means of the non-oxidative reactions of carbohydrate catabolism (e.g., the enolase reaction). The ATP can then be produced only slowly and in amounts insufficient to supply the energy requirements. In diabetes, the tissues and the red

Blood cells show diminished ATP [22, 23, 24]. Insulin, which restores the oxidative method of glucose catabolism, returns these values to normal and increases the rate of production of ATP [25]. The fact that the basal metabolic rate is normal in diabetes is irrelevant as regards the diminished synthesis of ATP, for the production of calories and the production of high-energy compounds need not be parallel reactions.

Special Biochemistry of Carbohydrate Metabolism in Diabetes

Impaired Breakdown of Glucose and its Sequelae

The production of energy by the breakdown of carbohydrates takes place in the same way, with minor variations, in all living things, including bacteria, plants, and animals. This basic process of life does not occur by a single chain of reactions, as formerly believed, but accomplishes its goal by means of several alternate pathways which guarantee the steady progress of the total process by alternate or substitute reactions as necessary.

The classical "glycolytic" pathway of catabolism of the hexoses in muscle to pyruvic acid or lactic acid was detailed by Embden and Meyerhof (Fig. 53). With the exception of the last step, this is identical with the alcoholic fermentation seen in yeast. This process was once considered the only or at least the most important pathway of glucose breakdown. At the same time, Martius, H. A. Krebs, and Ochoa described an accompanying mechanism of oxidation of pyruvic acid to carbon dioxide and water by the citric acid (tricarboxylic acid) cycle, which is also present in all living things (Fig. 54). The combination of the two pathways explained for the first time the total oxidative catabolism of carbohydrates in all its details.

It is only recently that a second ubiquitous pathway has been discovered by Dickens, Dische, Horecker, and Racker. This differs from the pathway of glycolysis in that it affects six molecules of hexose, of which one is completely oxidized; the other five are regenerated (Fig. 55). In bacteria and probably also in higher organisms there is a third mechanism, described by Doudoroff and Entner, in which there is a semi-oxidative shunt. The biological significance of this third pathway is uncertain, (Fig. 56). It is important to note that at least the Embden-Meyerhof (E-H) pathway and the Dickens-Horecker (D-H) cycle are both concerned, side by side, in the production of energy in most forms of life, although the amount of glucose catabolized by each pathway differs in different organisms and even in different cells or different functional states of the same organism.

Studies show direct oxidation of the glucose in the D-H cycle occurs to a greater degree than glycolysis by the Embden-Meyerhof pathway. The D-H cycle, accompanied by a CO_2 -fixing reaction, is the basis of the photosynthetic assimilation of carbohydrates in all green plants, one of the fundamental reactions in nature [26]. It is also the chief or only pathway of energy production in primitive forms of life, such as bacteria and the lower fungi [27, 28], as well as in the entire animal kingdom from sea-urchins, worms, and insects [29, 30, 31] to the mammals including man [32]. The great importance of the D-H cycle in carbohydrate metabolism together with the E-H cycle is shown by the fact that there is no form of life in which the D-H cycle is absent, although there are several without the E-M pathway (e. g., *Pseudomonas fluorescens*). In the mammalian body, the D-H cycle is almost completely absent in skeletal muscle and in the brain [33, 34, 35]. It accounts for approximately 20% of the

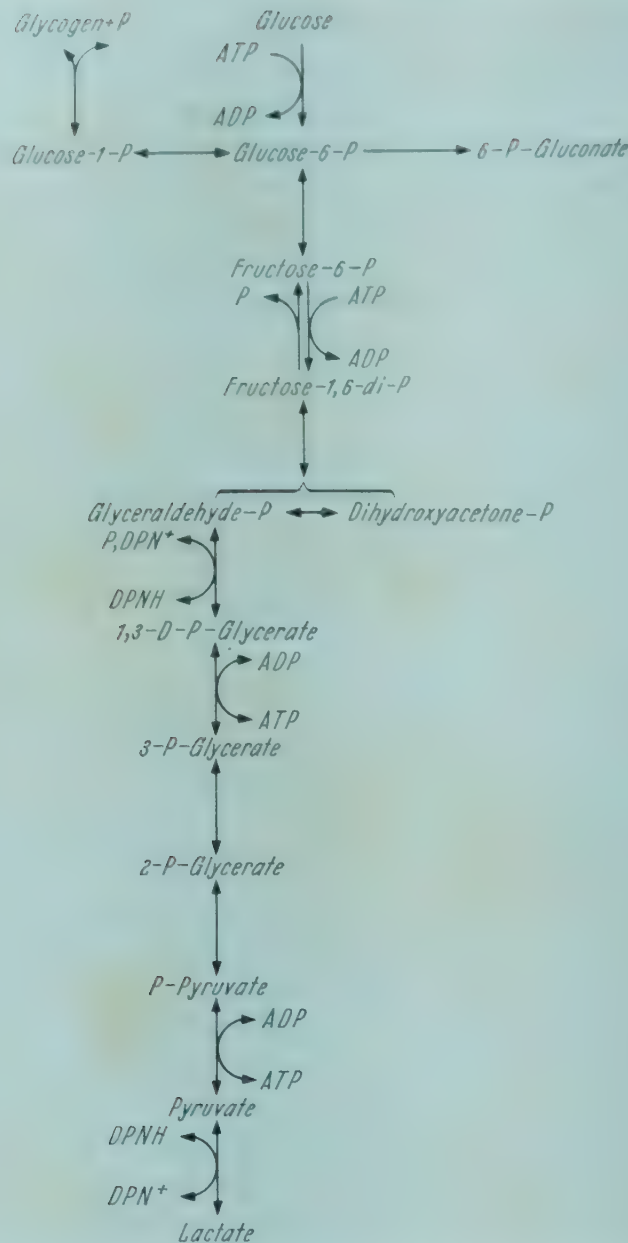


Fig. 53. Glycolytic pathway of catabolism of glucose (Embden-Meyerhof).

oxidative metabolism of carbohydrates in the kidneys [35], 35% in the cornea [36], and 50 to 70% in the liver, lungs, and spleen [34, 35]. In the adrenal glands, the lactating breast, and the lens of the eye, the D-H cycle is virtually the only pathway of production of CO_2 from carbohydrates [34, 37, 38]. There is some evidence that the D-H cycle is the main source of hydrogen for the endergonic synthetic processes, whereas the E-M pathway and the citric acid cycle supply the organism, not only with the necessary energy, but also with the carbon skeletons for amino acid and fatty acid syntheses.

Three different groups of workers independently and by different methods showed that *in diabetes there is diminished participation of the D-H cycle* in carbohydrate metabolism [40, 41]. In the diabetic liver, the contribution of the D-H cycle to oxidative degradation of glucose was reduced to less than half of normal, while there was an increase in the amount of lactic acid derived from the E-M pathway and oxidized to carbon dioxide [41 a]. Chaikoff and his group showed that both the production of carbon dioxide and the synthesis of fatty acid from glucose in the liver in alloxan

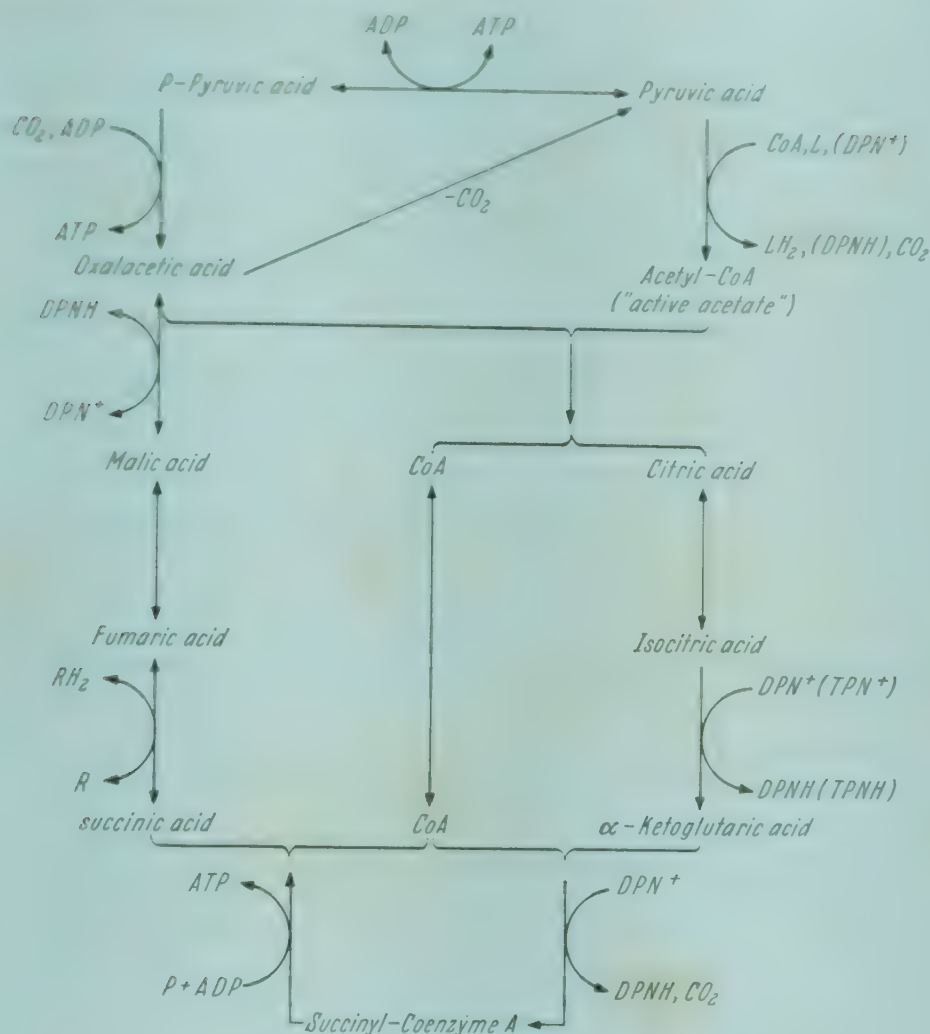


Fig. 54. The citric acid (tricarboxylic acid) cycle.

diabetic rats take place only by way of the E-M pathway, in contrast to normal; the amount of E-M catabolism is diminished, but to a lesser degree than the D-H cycle [41 b]. The quantitative aspects of these studies, however, are uncertain. The fundamental calculations in most of these studies are based on the different rates of oxidation of carbons 1 and 6 of glucose. The most important assumption of this type of method as regards metabolic measurements notwithstanding, the unlimited function of the citric acid cycle is not realized in all tissues, as has been shown in studies of glucose metabolism in the lens [670, 671] and the cornea [671]. However, the data show that in diabetes and in chronic starvation [35, 40, 41] there is a shift of oxidative catabolism of glucose in the liver from the D-H cycle to the E-M pathway and further to the citric acid cycle. These facts explain the *diminished ribonucleic acid content* of the tissues in diabetes [40] and in starvation [40, 42], since formation of ribose phosphate is a step in the degradation of glucose in the D-H cycle. There is a corresponding marked reduction of the activity of the enzymes of the D-H cycle in the diabetic liver, especially glucose-6-phosphate and 6-phosphogluconate dehydrogenases [40].

This metabolic defect first affects those tissues which derive a large proportion of their energy either directly or indirectly from the D-H pathway, especially the *liver*, the *adrenals*, and the *crystalline lens*; hence the occurrence in diabetes of disturbances of liver function, changes of secretory activity of the adrenal glands, and cataracts.

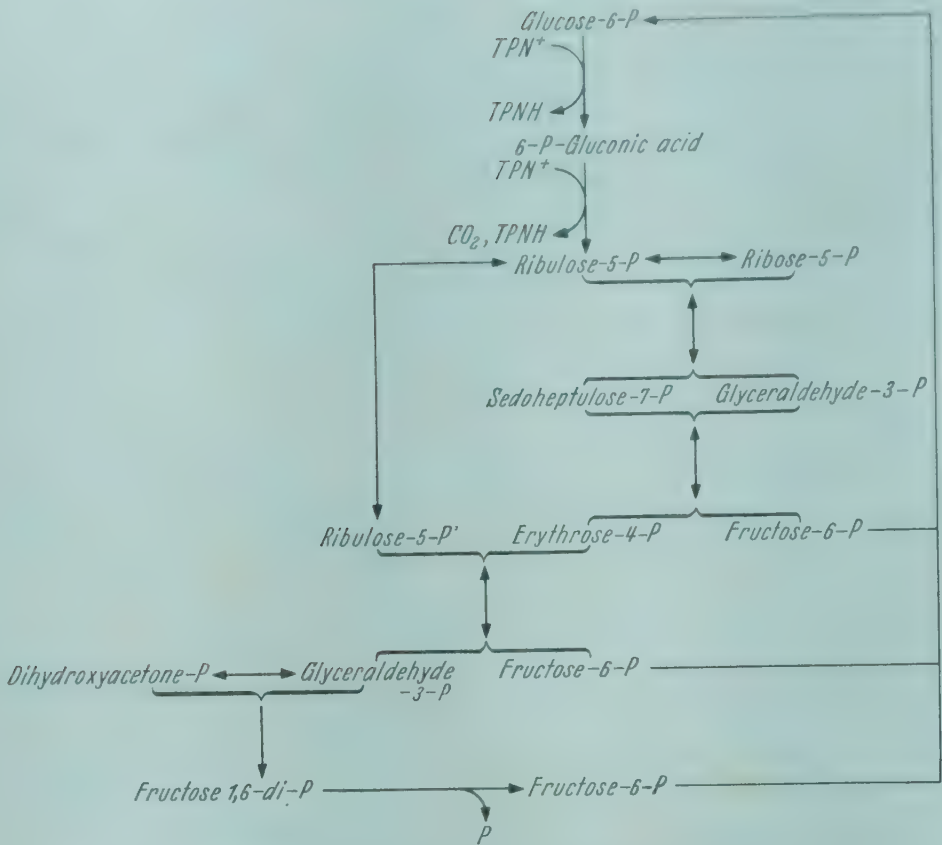


Fig. 55. The direct oxidative catabolism of glucose (Dickens-Horecker).

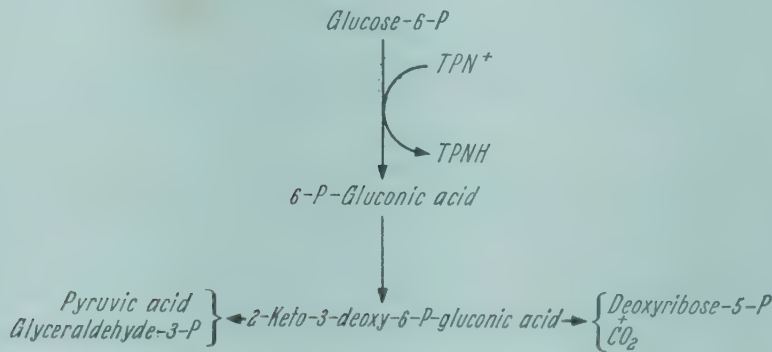


Fig. 56. Catabolism of glucose according to Doudoroff and Entner.

On the other hand, those tissues (e. g., muscles and the central nervous system) which normally derive their energy by the E-M pathway are not affected when the direct pathway of oxidation fails, so that their function is not impaired in uncomplicated diabetes. It is not clear whether the diminution of oxidative degradation of carbohydrates by the Dickens-Horecker pathway as found in diabetes is primarily related to a failure of hormonal control. When oxidation of carbohydrates is blocked in the adrenal glands where virtually only the D-H cycle is active, the coenzyme of dehydrogenation of the D-H cycle, TPN, which is normally present in its reduced form TPNH, may be converted to its oxidized form TPN⁺ because of lack of substrate hydrogen (p. 192). The result may be a hypernormal tendency towards a reaction by

means of which the steroid precursors of the adrenal cortical hormones are oxidized to the gluconeogenic/diabetogenic 11-hydroxy steroids such as cortisone (p. 232). The oxidative synthesis of these hormones within the adrenal cortex occurs only in the presence of TPN⁺ [43]. These facts would suggest a causal relationship between the disturbed breakdown of carbohydrates seen in diabetes and the simultaneous overproduction of glucose which is mediated by the adrenal cortex. The enzymic and hormonal disturbances in the adrenals in diabetes can thus be integrated into a single concept.

The ultimate causes for the inhibition of direct oxidation of carbohydrates in diabetes without concomitant and equal diminution of glycolysis are poorly understood. However, it is certain that an enzymic failure is responsible, which affects both of the catabolic pathways at the same time. Both the oxidative and the glycolytic pathways begin with glucose-6-phosphate, and it is reasonable to postulate that the synthesis of this ester from free glucose is blocked in diabetes. There is a branching of glucose metabolism at this point (p. 206). The half-value of saturation of the two enzymes concerned (E-M or D-H pathways) may occur at different concentrations of the substrate, so that the unequal effects of the two pathways of degradation can be explained by inhibition of production of substrate at the branching point. The Michaelis constants of the enzymes concerned have not been determined exactly, so that the truth of this concept has not yet been established. However, numerous observations have shown that the synthesis of glucose-6-phosphate (*the hexokinase reaction*) is markedly reduced in most tissues in the diabetic. Quantitative studies with radioactive glucose [119] have shown that 75 to 90% less glucose-6-phosphate is synthesized from glucose in liver slices of diabetic rats than in normal rats per unit time.

The analysis of the significance of this phenomenon is difficult, for the phosphorylation of glucose is not complete unto itself in the homogeneous milieu of the tissue fluids, but is coupled with the *passage of glucose* from the extracellular space *into the interior of the cells*. The phosphorylating enzyme hexokinase is not in solution within the tissues, but is adsorbed onto the intracellular surface of the cell membrane [44, 45, 228]. This fact apparently underlies the special physiological significance of this initial reaction of general carbohydrate metabolism, by means of which plasma glucose is retained within the cells as glucose phosphate, so that the catabolic enzymes, which are present only within the cells, can function. The impairment of this reaction in diabetic tissues thus paralyzes carbohydrate metabolism at its very beginning. It is still uncertain, however, if the defect which causes the impairment affects the purely enzymic process of phosphorylation or the more physico-chemical process of the *passage of glucose through the cell membrane*.

Cori and his coworkers in 1945 were the first to show the diminished phosphorylation of glucose in diabetic tissues. They explained the diminution by an inhibition of the enzyme hexokinase by the antagonistic "diabetogenic" hormones of the anterior pituitary or the adrenal cortex [46, 47]. This concept has not yet been entirely confirmed, for impaired hexokinase activity has not been distinctly shown either in human [48] or experimental [49, 50] diabetes. There are certain facts which speak against a reduced hexokinase activity in the tissues of diabetics. Thus, the inhibition caused by extracts of the anterior pituitary and the adrenal cortex in Cori's experiments when cell free preparations of hexokinase were used was not definitely influenced by insulin [51-53]. On the other hand, the action of insulin is quite independent of the events which influence the hexokinase activity (e. g., the concentration of ATP in muscle) [54]. It is thus unlikely that the primary distur-

bance of diabetic metabolism is in the hexokinase reaction itself. It is more likely that the impairment of phosphorylation of glucose, which superficially simulates inhibition of hexokinase, is merely one phenomenon of a more fundamental process, the blocking of the entry of glucose into the cell. R. Höber suggested such a mechanism as the etiology of diabetes as long ago as 1914 [55]. In actual fact, the intracellular concentration of glucose falls to zero in alloxan diabetes and in starvation, while insulin increases the concentration above normal [56].

The fundamental action of insulin is probably a specific "active" transfer of glucose across the cell membrane. The energy requirement of this action is supplied by an increase of oxidative phosphorylation (p. 215). Hence, all forms of diabetes which are due either directly or indirectly to a deficiency of insulin have as their fundamental and primary defect an inhibition of the transfer of glucose from the extracellular space into the cell. In alloxan diabetes, for example, the transfer of glucose into the cell is delayed and is returned to normal by insulin [57]. Thus, the intracellular glucose would be reduced in diabetes, and this is actually the case, while if the transfer of glucose were intact and inhibition of hexokinase were primary, the intracellular glucose would be increased. The inhibition of transfer prevents the contact of glucose with the hexokinase which is fixed to the inner side of the cell membrane, thus simulating inhibition of hexokinase. Thus, the transfer of glucose is the limiting factor which controls the degree of uptake and utilization of glucose [57].

Glucose transfer and hexokinase activity are related to each other in glucose metabolism, but recent technics have permitted separate studies of the two events [58, 59] and have also shown that it is the transfer mechanism which is the central defect in insulin-deficiency diabetes. The nature of this highly specific disturbance, however, is poorly understood (p. 214). Some evidence has been provided by the observation [60] that there is a close relationship between the ability of glucose and its structural analogs to pass through cell membranes, and their ability to serve as the substrate for the enzyme mutarotase, which converts them into an easily absorbed form [66]. From the standpoint of the economy of carbohydrate metabolism, the collaboration of glucose transfer across the cell membrane and hexokinase activity is of great importance. On the one hand, only glucose, and not glucose-6-phosphate, passes through the cell membrane, and on the other hand the hexokinase reaction which converts glucose into glucose-6-phosphate is an irreversible reaction. Hence, this reaction is apparently a reaction which prevents the intracellular glucose from leaving the cell, so that it cannot avoid breakdown by the intracellular enzymes. In diabetes, all the catabolic mechanisms which affect glucose are impaired as a result of the inhibition of the entry of glucose into the cells and lack of oxidative mechanisms in the extracellular space. This is not true, however, for the red cells or the cells of the central nervous system: the surfaces of these cells are permeable to glucose even in the presence of insulin deficiency [214].

The blocking of glucose entry into the cells has two basic effects: (1) *an increase of the extracellular glucose pool*, which can rise to four times normal in diabetes, together with an *increase of the turnover time of body glucose* [61 b]; and (2) *decrease of the overall oxidation of glucose within the cells*, without decrease of the individual enzymic steps of glucose breakdown in diabetes. Thus, no direct disturbance of the catabolic pathway of glucose and lactic acid to "active" acetate has been found in pancreatic and alloxan diabetes [62-64]. Isotope studies of the formation of carbon dioxide from carboxyl-labelled lactate in normal and diabetic rats have shown that the decarboxylation of pyruvic acid in diabetes may be increased as compared with the normal [65].

In addition, the utilization of glycerol (from fats and phosphatides) by way of triose phosphate and pyruvic acid is normal in diabetes [67]. The utilization of the products of carbohydrate metabolism is first inhibited at the stage of active acetate, so that one must assume an additional specific defect at this point of the enzyme chain of the E-M pathway. In diabetes there is marked impairment of a number of reactions, including acetylation [68], the incorporation of acetate into citrate [70] and fatty acids [71-73], and the oxidation of acetate to CO_2 by way of the citric acid cycle [69], probably because of an insufficient supply of the reduced form (SH-form) of coenzyme A which is necessary for these reactions. The transient reduction of utilization of pyruvate in diabetic tissues is the indirect result of accumulation due to diminution of acetate turnover. In addition to this biochemical abnormality which affects acetate and thus threatens the synthesis of citric acid [70], another defect is of importance. In the oxidation of glucose, which is delayed and inhibited by a deficiency of ATP, the oxalacetic acid which comes chiefly from phosphopyruvic acid and CO_2 is also present in reduced amounts, so that the synthesis of citric acid in diabetes is impaired by a second mechanism.

As a result of the deficiency of oxalacetic acid, the active acetate which is produced in large amounts in the intermediary catabolism of fatty acids cannot be metabolized in the normal fashion by way of the citric acid cycle. It first accumulates and later is converted in the liver to acetoacetic acid because of the reversible nature of acetoacetic acid splitting [74]. This condensation reaction is the opposite of the mechanism which occurs physiologically. First, two molecules of active acetate condense to give one molecule of "active" acetoacetic acid and one molecule of coenzyme A, and then the "active" acetoacetic acid is broken down spontaneously to free acetoacetic acid and coenzyme A [75]. As a result of this continuing reaction, new amounts of "active" acetate constantly flow into this synthesis, so that ultimately all the unutilized active acetate is converted in the diabetic body to acetoacetic acid. *This is the fundamental reaction which leads to diabetic acidosis.* The acetoacetic acid is acted upon by enzymes in two ways, giving β -hydroxybutyric acid by reversible hydration on the one hand, and acetone by decarboxylation on the other. Acetoacetic acid, β -hydroxybutyric acid, and acetone are known collectively as acetone bodies or ketone bodies. Acetoacetic acid is formed from the "active" acetate found in intermediary fat metabolism and also from the "ketoplastic" amino acids leucine, phenylalanine, and tyrosine [76 a].

The ketone bodies threaten the diabetic individual in two ways. In the first place there is danger of acidosis, since acetoacetic acid and β -hydroxybutyric acid are relatively strong acids (pK_a 3.6 and 4.8). In the second place, the ketone bodies are lipid-soluble substances which pass readily through all cell walls and exert a narcotic effect within the cell [76 b]. The production of the ketone bodies is governed by a complicated endocrine mechanism, the details of which are not known (p. 236). Both the growth hormone of the anterior pituitary and the corticosteroids of the adrenal increase the production of ketone bodies. These hormones show increased activity in diabetes but not in starvation (with the exception of late stages and of hunger diabetes), so that the production of ketone bodies is much more pronounced in diabetes than in starvation.

In the normal, the active acetoacetic acid formed is directly converted to active β -hydroxybutyric acid. In diabetes and in carbohydrate-free regimes, however, the active acetoacetic acid is first converted to free acetoacetic acid and only then hydrated to free β -hydroxybutyric acid. This difference is probably due to the different

characteristics of the two separate enzymes which reduce the active and free forms of acetoacetic acid. These two enzymes are active at two different redox potentials. In addition, the shift of the redox potential in a positive direction, which is characteristic of diabetes, permits hydration only of the free form of acetoacetic acid.

The production of ketone bodies in diabetes is basically a result of the disturbed catabolism of glucose and the resulting deficiency of oxalacetic acid. It can therefore be decreased by any measures which return glucose catabolism to normal, including the administration of insulin (p. 213) or the increase of all substances which lie between glucose and oxalacetic acid in the catabolic pathway. An increase of these substances in the tissues results, because of mass action, in an increased flux through the various enzyme reactions involved in catabolism of glucose, so that the formation of oxalacetic acid is increased. Among such "*antiketogenic*" substances are the monosaccharides of the cells and their phosphoric acid esters, as well as pyruvic acid, lactic acid, and the acids of the citric acid cycle [77]. A number of other compounds which can enter glucose catabolism by a side reaction, such as sorbitol (p. 210) and the glucoplastic amino acids (Table 52) also have weak antiketogenic activity.

The increased synthesis of cholesterol which occurs in diabetes is also due to the accumulation of active acetate with subsequent recondensation to acetoacetic acid [78, 79]. The well known increase of serum and tissue cholesterol of human diabetes has been verified in animals by isotope technics. Cholesterol and fatty acids are formed in animals in the same way from active acetate, but the pathways of synthesis of the two groups of substances are different from the very beginning: that of cholesterol, in contrast to that of the fatty acids, occurs by way of free acetoacetic acid [80, 81]. In diabetes, more or less acetoacetic acid is formed depending on the severity of the individual case. In contrast to active acetoacetic acid, this acetoacetic acid cannot be used for the synthesis of fatty acids, and its appearance is partly responsible for the impaired synthesis of fats from carbohydrates and amino acids. On the other hand, this acetoacetic acid can serve in its entirety as a substrate for the synthesis of cholesterol [81, 82]. Enzymic condensation with an additional molecule of active acetate [672] in the liver gives β -hydroxy- β -methylglutaric acid [83]. This compound already has the branched carbonskeleton of the isoprene building block mevalonic acid, which is formed from β -hydroxy- β -methylglutaric acid and then undergoes several polymerizations, via the farnesine skeleton, to form squalene and then cholesterol.

Except for the initial reaction, the formation of hydroxy-methyl-glutaric acid, this entire process requires no additional activation by coenzyme A [88]. This fact, together with the fact that it stems from the increased acetoacetic acid of the diabetic (which cannot be used for other syntheses), explains the increased production of cholesterol seen in diabetes. In addition, cholesterol synthesis requires far less hydrogen than does the synthesis of fatty acids, so that cholesterol is more readily synthesized than fat in the hydrogen-poor diabetic body (p. 192). Thus, 32 molecules of hydrogen are needed for the conversion of 18 molecules of active acetic acid into 2 molecules of stearic acid, while only 4 molecules of hydrogen are needed for the conversion of 18 molecules of active acetic acid into 1 molecule of cholesterol. In the diabetic, thus, the synthesis of lipids is shifted towards cholesterol at the expense of fatty acid synthesis, while insulin corrects the situation and allows increased synthesis of fatty acids (p. 213). Insulin acts by increasing the degree of hydration of the coenzymes and the synthesis of high-energy compounds (active fatty acids). The significance of the increased formation of cholesterol in diabetes is two-fold: (1) there

is danger of atheromatous and sclerotic changes in the blood vessels and tissues, and (2) the increased cholesterol allows increased production of corticosteroids (hormones which are derived from cholesterol), which increase gluconeogenesis and thus have an unfavorable effect on the metabolism of diabetes.

Within the citric acid cycle itself, no specific enzymic block of glucose oxidation has been found in diabetes. The diminution of the acids of this cycle in the tissues of alloxan diabetic animals [89] is merely a secondary result of the disturbed incorporation of active acetate into citric acid, the initial step of the citric acid cycle. Thus, disturbed breakdown of glucose has been shown for certain in diabetes at only two points, the transfer of glucose to the interior of the cell, and the stage of active acetate. Under special conditions, however, it may be possible to produce a *disturbance of metabolism similar to that seen in diabetes, by experimental blocking of each individual step of the E-M pathway and the citric acid cycle*. Thus, the E-M glycolytic pathway can be interrupted at the stage of triose phosphate by means of iodoacetic acid [90, 91], a substance which eliminates the SH-groups of triose phosphate dehydrogenase. The same pathway can also be interrupted at the stage of 2-phosphoglyceric acid by means of fluoride, which inactivates enolase [92, 93]. Both these artificial devices lead to the same result, hyperglycemia and ketonemia — i. e., a diabetes-like state of metabolism. If the utilization of citric acid itself is inhibited by means of fluoracetic acid, the interruption of oxidative catabolism of glucose also results in diabetes [94, 95]. The same condition occurs when interruption occurs at the stage of succinic acid by the use of malonate [96].

The action of iodoacetic acid described above is merely a special case of a general law, that the introduction of SH-binding or SH-oxidizing substances into the body leads to diabetes by inactivating the SH-groups of the mercaptoenzymes involved in carbohydrate metabolism (p. 192). Disulfides act in this way [97]. Greater activity is shown by oxidizing substances such as alloxan [98, 99], chromate [100], dehydroascorbic acid [101], and riboflavin in large doses [102]. At least part of the diabetogenic action of alloxan is due to the fact that it causes oxidative binding and breakdown of the sulfhydryl groups in the tissues [103]. The same is true for epinephrine, which causes a fall of the SH content of the liver and kidneys [104]. The diabetogenic effect of such oxidative inhibition of active SH groups is closely related to the finding that diabetics always show an increase in the $\frac{\text{DPN}^+}{\text{DPNH}}$ ratio and a shift of the redox potential in a positive direction. These changes result in a stoppage of the catabolism of glucose and therefore in a deficiency of available hydrogen. The immediate result is a shift from synthesis of fatty acids to synthesis of acetone and cholesterol. The secondary impairment of the mercaptoenzymes involved in carbohydrate metabolism can lead to a vicious circle in the events of diabetic metabolism.

The degree to which the direct oxidative pathway of glucose catabolism (D-H cycle) also shows enzymic blocks in diabetes is not completely known. However, such blocks have been shown for the two initial steps of the pathway, the synthesis of 6-phosphogluconic acid and of ribulose-5-phosphate (p. 199). As has been discussed, the *D-H pathway in diabetic metabolism is lessened* in contrast to the Embden-Meyerhof-Krebs pathway. A third pathway of breakdown of glucose-6-phosphate which is closely related to carbohydrate metabolism is also blocked in diabetes. This is the *amination of glucose* to the amino sugars glucosamine and chondrosamine which are found in the form of polysaccharides [105]. Recent isotope studies have shown that the synthesis and renewal of the mucopolysaccharides and chondropolysaccharides

are reduced by 50 to 70% in the diabetic. This finding helps explain the poor resistance of the skin and connective tissues of diabetics and the resulting tendency to infection and gangrene. The impaired synthesis of the amino sugars in diabetes is partly due to the inability of glucose to pass into the cells of the body. In addition, however, it is due to the unavailability or diminished supply of L-glutamine which is necessary for this synthesis [106].

The direct oxidation of glucose to *glucuronic acid*, which is probably an "overflow" mechanism, is increased in diabetes [107].

The synthesis of protein is also diminished in diabetes. This decrease is the indirect result of the decrease of available energy which in turn is due to the impairment of carbohydrate oxidation. The synthesis of peptides and proteins from amino acids requires energy, which must be supplied by the oxidation of carbohydrates. Hence, the synthesis of proteins from amino acids is diminished in human and experimental diabetes [108, 109], and the level of free amino acids in the plasma rises [110]. The reduced synthesis of protein leads to a loss of protein and a negative nitrogen balance [111].

The synthesis of glutathione from glycine is also disturbed in diabetes [112]. Insulin corrects all these defects and thus spares protein because of its ability to retain glucose within the body cells, where oxidation of glucose and production of energy occur. The result is to supply the energy necessary for the synthesis of peptides and proteins. It should be noted that the impairment of protein synthesis due to diminished oxidation of glucose when insulin is lacking is difficult to distinguish in its sequelae from the increased destruction of protein due to the adrenal corticosteroids (p. 235).

Increased Gluconeogenesis in Diabetes

Soon after Külz (1875), B. Naunyn in 1893 proposed the "overproduction" theory of the pathogenesis of diabetes, according to which the basic disturbance in diabetes is the synthesis of glucose from non-carbohydrate precursors, especially proteins. Support was lent to this theory in the first decades of the twentieth century by G. Lusk, Lüthje, and Janney. These authors believed that such gluconeogenesis occurs primarily from protein precursors, but Geelmuyden in 1923 suggested that the chief source of newly-formed glucose in diabetes is fat. However, this latter theory was rejected by other clinicians, notably Thannhauser. No investigators were able to establish Naunyn's overproduction theory completely by any experimental means, and it remained for isotopic techniques to detail the metabolic processes in diabetes. Such techniques have succeeded in differentiating the type and extent of catabolic and turnover processes in the diabetic body and have shown that, in addition to the diminished oxidation of glucose in human and experimental diabetes, there is also gluconeogenesis. Both of these processes are responsible for the hyperglycemia and glycosuria of diabetes [1, 113] (Tables 50 and 51). Thus, recent studies with the glucose antagonist 2-deoxyglucose have shown that the metabolic defects which cause impaired transfer of glucose across the cell membrane and impaired breakdown of glucose are insufficient in themselves to produce diabetes. This glucose antagonist competitively displaces glucose and thus inhibits the entry of glucose into the cells, blocks the intracellular breakdown of glucose, and blocks the action of insulin on the oxidation of glucose [114]. The resulting abnormality resembles insulin-deficiency diabetes and is due to the fact that 2-deoxyglucose induces all the typical disturbances of metabolism found in spontaneous diabetes. Despite this, deoxyglucose ad-

Table 50. The carbohydrate metabolism of liver slices of normal rats, rats with pancreatic diabetes, and rats with alloxan diabetes under conditions of normal or impaired adrenal function. [from (113)]

No.	Number of rats	Previous management	Glycogen					Glucose		
			Initial value	Synthesis from		Glycogenolysis	Balance	Uptake	Output	
				glucose and pyruvate	unknown sources				Total	Portion from gluconeogenesis
			a	b		c	b - c	d	e	e - c
1	6	f normal	149.5	50.3	0	35.3	+ 15.0	61.0	69.0	33.7
2	5	f A	125.2	6.4	0	44.2	-37.8	34.2	158.0	113.8
3	1	f P	140	7.8	0	40.8	-33.0	4.0	92.0	51.2
4	5	f adr.	55.7	26.7	2.5	7.8	+ 21.4	53.0	67.4	59.6
5	6	f A and adr.	47.6	13.8	0.2	12.5	+ 1.5	23.5	76.2	63.7
6	6	n normal	3.3	22.0	0	0	+ 22.0	33.6	39.6	39.6
7	8	n A	49.5	?		?	-17.1	16.8	73	?
8	2	n P	101.0	4.2	0	20.0	-15.8	21	112	92

The medium has a pH of 7.4 and contains in mM liter: K⁺ 110, Mg⁺⁺ 20, Ca⁺⁺ 10, bicarbonate 40, Cl⁻ 90, pyruvate 40, and glucose 20. The labelled substrate included glucose-1, 2, 3, 4, 5, 6-C¹⁴ and α-C¹³-pyruvate. All values are averages and are expressed as micromoles of glucose or pyruvate per gram of fresh liver. The duration of each experiment was 90 minutes. Abbreviations: A = alloxan diabetes; P = pancreatic diabetes; adr = adrenalectomy; n = starved animal (fasting for 18-24 hours); f = fed animal.

ministration is not followed by clinical diabetes with hyperglycemia and glycosuria. This startling contradiction indicates that some additional metabolic defect must be responsible for the development of human and experimental diabetes; i. e., together with the impaired utilization of glucose, this defect produces clinical diabetes. This additional defect is, indeed, increased gluconeogenesis.

The increased production of glucose is accomplished by several methods and is further intensified by the accumulation of glucose which results from the diminished breakdown of glucose. The first important mechanism in the diabetic is activation (or,

Table 51. The metabolism of glucose in the dog as determined with glucose-1-C¹⁴ (from Feller Chaikoff, Strisower and Searle)

Dog, 6-7 kg	Glucose pool (in equilibrium with blood glucose), grams	Rate of oxidation (formation of CO ₂ from glucose) grams per hour	Turnover-time of glucose pool hours	Rate of turnover of the glucose pool		Proportion of exhaled CO ₂ which comes from glucose %
				grams per hour	Portion oxidized to CO ₂ %	
Normal	3-4	1.7-2.3	1.2-1.7	2.3	80-95	50-70
Pancreatic diabetes	12-21	0.5-0.8	4-5	3-4	20	10-20
Pancreatic diabetes plus insulin	5-6	1.5-2.0	1.5-2.0	3-4	50	45

perhaps, loss of inhibition (1) of the *glucose-6-phosphatase* of the liver. Since this enzyme converts glucose-6-phosphate to free glucose, it represents the immediate mechanism which synthesizes glucose [116]. Under normal conditions, the activity of this enzyme is small and is precisely adapted to the particular energy requirements of the peripheral tissues. Normally, apparently, the action of glucose-6-phosphatase is partly masked by a physiological inhibitor [116]. In diabetes and in prolonged starvation, the activity of this enzyme in the liver is increased two- to three-fold [117]. The overfunctioning of this enzyme could simulate reduced hexokinase activity, for the reaction catalyzed by glucose-6-phosphatase is the reverse of the hexokinase reaction

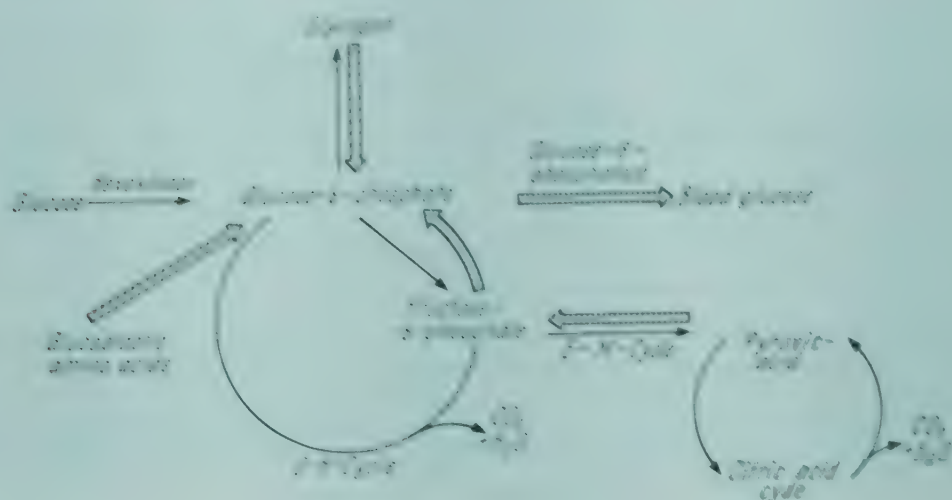


Fig. 57. The central position of glucose-6-phosphate in the catabolism of carbohydrates.

and leads to the same metabolic result as hexokinase inhibition. The activation of glucose-6-phosphatase in diabetes is a good example of the control of enzymes by hormones: activation occurs either because of a deficiency of insulin, a substance which inhibits the enzyme [116], or because of an excess of cortisone, which increases the activity of the enzyme [118]. The increased activity of glucose-6-phosphatase in the diabetic liver is of fundamental importance in the pathophysiology of the disease. It is responsible for an important segment of the diabetic disturbance in metabolism in the liver, which is the only tissue, except for the kidneys, which contains the enzyme. This is a strong argument against all hypotheses which state that diabetogenic substances act directly on the peripheral tissues.

The increased activity of glucose-6-phosphatase explains a number of phenomena characteristic of diabetic metabolism. Its direct effect is the diversion of carbohydrate metabolism from the synthesis of glycogen to the increased production of glucose (glycogenolysis). The stage of glucose-6-phosphate in carbohydrate metabolism can proceed in 4 different directions (Fig. 57). Normally, when adequate glucose is present, glucose-6-phosphate is largely converted to glycogen; in diabetes, however, most of it is broken down instead to form free glucose. This latter reaction is due to the increased activity of glucose-6-phosphatase plus simultaneous decrease of the Embden-Meyerhof-Krebs and Dickens-Horecker pathways. The glucose-6-phosphate is replaced not only by the preformed glucose of the portal blood and by mobilization of preexisting glycogen stores, but also by the intermediary products of the two

catabolic pathways, which accumulate and are resynthesized to carbohydrate according to the law of mass action. Thus, radioactive glycerol and pyruvate provide less glycogen and more free glucose in the diabetic liver than in the normal [67]. The activated glucose-6-phosphatase thus has two effects: (1) *increased glycogenolysis*, also shown in liver slices in alloxan diabetes [113] (Table 50); and (2) *increased synthesis of glucose* from the low-molecular intermediary products of carbohydrate metabolism.

In addition, the increased activity of glucose-6-phosphatase in the diabetic liver also induces *true synthesis of glucose* from protein and, to a small degree, from fatty acids. Gluconeogenesis from protein has been known for years, and has been measured clinically by the ratio of glucose (dextrose) in the urine to nitrogen in the urine (D:N). Modern isotopic techniques have permitted quantitative analysis of this process. Thus, in the liver of alloxan and pancreatic diabetic animals, gluconeogenesis from protein is two to four times the normal [113] (Table 50), and the same results obtain in the intact animal [121]. In the diabetic body, this is an essential process for the maintenance of the energy supply and serves to increase the blood glucose and therefore to increase the blocked transfer of substrate in the Embden-Meyerhof-Krebs chain of glucose catabolism. As Table 52 shows, the synthesis of glycogen is not completely abolished in diabetes. As long as some synthesis of glycogen is possible, gluconeogenesis results not only in overproduction of glucose, but also in an increased amount of liver glycogen. The relatively high liver glycogen of alloxan diabetic animals, which is especially striking in starvation in contrast to the small amount of glycogen of normal livers (Table 50), must be explained by the marked increase of gluconeogenesis in diabetes [113, 122, 123, 124], which in turn is due to an increased production and secretion of adrenal cortical hormones. Adrenalectomy or hypophysectomy, the latter by eliminating ACTH, restores liver glycogen to normal [120, 125].

True gluconeogenesis occurs by conversion of proteins (amino acids) to glucose and is therefore accompanied by breakdown of protein and a negative nitrogen balance.

Table 52. The glucoplastic amino acids

Amino acids	Intermediate products formed in the conversion to carbohydrates
Glycine	Glycol aldehyde (?), serine → pyruvic acid
Alanine, Serine	Pyruvic acid
Valine	Propionic acid → pyruvic acid
Isoleucine	Active acetate + pyruvic acid
Methionine	Homoserine, α-ketobutyric acid
Threonine	Glycine + active acetate
Aspartic acid	Oxalacetic acid
Glutamic acid	α-ketoglutaric acid
Proline, Histidine, Lysine	} - glutamic acid → α-ketoglutaric acid
Arginine (via ornithine)	
Cyst (e) ine	Pyruvic acid

findings which are present in both human and experimental diabetes [126-128]. The substrates are the so-called "glucoplastic" amino acids, which form glycogen in the normal and extra glucose in the diabetic body. These amino acids undergo deamination or transamination and form intermediary products of the Embden-Meyerhof-Krebs pathway of carbohydrate catabolism, which are then converted to glucose (Table 52).

In accordance with this fact, the transaminase activity of the diabetic liver is higher than normal [129]. The nitrogen released when glucose is synthesized from protein is excreted as urea. Glycine is a special case: it can be converted to liver glycogen or extra glucose [130], but it also indirectly stimulates the adrenal cortex to a general increase of gluconeogenetic processes [131, 132, 133].

Gluconeogenesis from fat is quantitatively less important. The glycerol portion of the fats must be considered separately in this process, since glycerol itself is a derivative of carbohydrates and is converted to glucose to the same extent in the diabetic as in the normal person (p. 199). The problem of the conversion of fatty acids into glucose is much more complex. For years there was considerable disagreement as to whether carbohydrates can be synthesized from fatty acids, but isotope studies have left no doubt that such synthesis occurs in diabetes. Normally, the conversion of fatty acids into glucose takes place only by way of the citric acid cycle, by condensation of the C_2 moiety of active acetate which is produced in the breakdown of fatty acids, with oxalacetic acid [134]. There is the additional possibility that some fatty acids in the diabetic body can be converted by way of acetoacetic acid and acetone to pyruvic acid, and then by reversal of glycolysis can circumvent the citric acid cycle and produce glucose. The formation of pyruvic acid from acetone by way of propanediol-1-phosphate has been established by isotopic studies in mammalian liver [135, 136]. Each accumulation of the lower catabolic products of the E-M pathway from side-reactions, which is linked to an increased "stationary" concentration of the substance (e. g., the formation of pyruvic acid from acetone), must also increase the tendency to gluconeogenesis by causing a reversal of glycolysis by mass action. The results obtained in studies of the incorporation of carbon atoms of radioactive palmitic acid into glucose in diabetic dogs [137] do not contradict these statements. The demonstration of gluconeogenesis from fatty acids via acetone would finally give confirmation to the old ideas of Geelmuyden.

Metabolism of Fructose in Diabetes

The importance of fructose in cellular metabolism is based on the fact that fructose-6-phosphate and fructose-1,6-diphosphate are obligatory intermediate products in the catabolism of glucose (p. 195). Free fructose is found in the animal body only in the seminal fluid, in the prostate, in the amniotic fluid, and in fetal blood. Its presence is the result of a synthesis which takes place in the seminal vesicles and in the placenta at the expense of the glucose of the blood. The fact that the fructose content of seminal fluid in diabetes is elevated in proportion to the level of the blood sugar and is immediately returned to normal by insulin [138] indicates a close relationship between sperm fructose and blood glucose, and shows that fructose takes part in the events of diabetic metabolism. At first this fact seems to have little significance, for free fructose is present only in a few highly specialized tissues and their secretions, and the phosphoric acid esters of fructose which are present at other sites of the body can never be converted to free fructose. This, of course, is the opposite of the situation with regard to glucose and its phosphoric acid esters. In the diabetic, however, fructose is of special interest because this sugar, which is closely related to glucose, is utilized more or less completely - in contrast to glucose itself. Hence, even in diabetes, fructose has found an important therapeutic role as a "substitute carbohydrate" which supplies energy. It must be concluded that the catabolism of fructose in animal and man is different from that of glucose and does not depend on insulin. It

has been shown that *fructose metabolism differs from that of glucose in two respects, namely, in its relationship to insulin, and in the unique makeup of its reaction chain.*

Glucose can enter the tissue cell only in the presence of insulin, and so cannot function as a source of energy for cells if insulin is absent. Fructose, on the other hand, can pass from the extracellular space into the tissue cell without the mediation of insulin (at least in the chief organ of fructose metabolism, the liver) [139]. As a result, in both human and alloxan diabetes the utilization of fructose is complete [140, 141, 142].

At first, the fact that fructose is oxidized independently of insulin was explained on the basis that fructose was phosphorylated by fructokinase, which is insulin-insensitive, in contrast to hexokinase [140-144]. However the demonstration that insulin does not act on the phosphorylating kinases but rather on the transfer of glucose through the cell membrane has made this phosphorylation explanation of the preferential oxidation of fructose unlikely, and has substituted the membrane theory [61 a, 139]. In actual fact, however, fructose does not contain the chemical groups which are essential in order that its entry into the cell be influenced by insulin (p. 214).

The excellent utilization of fructose in diabetes was well known to the older clinicians K  lz (1874), Minkowski (1893), and von Noorden (1917), and is demonstrated by a series of biochemical phenomena which clearly distinguish the metabolism of fructose from that of glucose. Thus, the administration of glucose to diabetics has little effect on the blood level of pyruvate because of the impaired breakdown of glucose. Fructose, on the other hand, increases this level both in normals and especially in diabetics. Fructose disappears equally rapidly from the blood of normals and diabetics and supplies equal amounts of citric acid [146]. Even in severe diabetic acidosis, fructose is well metabolized and has a pronounced antiketogenic action (p. 200) [147, 148], showing that this sugar is metabolized in the citric acid cycle. However, the increased conversion of fructose to glucose, which occurs to a greater degree as the diabetes becomes worse, and takes place in the wall of the intestine and in the liver [149, 145], must be considered as an abnormal factor which increases the requirements for insulin [149, 150]. Hence the therapeutic use of fructose in severe diabetes and diabetic coma must be undertaken with care.

It is well known that in mammals, including man, fructose is converted to liver glycogen by way of glucose-6-phosphate, and that fructose forms glycogen more rapidly and to a greater extent than glucose. The same observations have been made in vitro in studies of liver slices [119, 151, 152]. In diabetes, the synthesis of glycogen from glucose is reduced by 95% or more, while that from fructose, although decreased to one-fourth the normal, can still be readily measured (Table 53). The decrease is

Table 53. Metabolism of fructose in liver slices of normal and diabetic rats [119]

		Uptake of fructose	Glycogen	Glucose	CO ₂	Lactate
			from fructose			
A	normal	158	35.5	56	17	49
	diabetic	151	8.5	75		24
B	normal	96	18.3	31	13	
	diabetic	99	2.5	69	7.3	

A: medium of fructose 30 mM/liter
B: medium of glucose plus fructose, each 15 mM/liter
The values are μ M per gram of liver in 90 minutes.

related to the increased activity of glucose-6-phosphatase which results in increased gluconeogenesis from glucose-6-phosphate in the diabetic liver. The increase of muscle glycogen after the administration of fructose is probably mediated by the conversion of fructose to glucose in the liver, with subsequent conversion of the glucose to glycogen in the muscles [153]. The oxidation of fructose to carbon dioxide in the diabetic liver is not normal but is reduced by only about one-third under conditions which cause the oxidation of glucose to fall by two-thirds [119].

The biochemical studies of the behavior of fructose in the diabetic body thus confirm that fructose metabolism proceeds along other pathways than that of glucose. In the presence of insulin, glucose is metabolized in the same way in all tissues, according to the E-M and the D-H mechanisms. Only the relative proportion of the two pathways may vary. Fructose, however, undergoes an entirely different metabolism in the liver and the muscles. In the liver, fructose is converted to fructose-1-phosphate, which is then split by the enzyme phosphofructaldolase to dihydroxyacetone-phosphate and D-glyceraldehyde. The latter can either be converted by way of glyceric acid to pyruvic acid or can be converted by the phosphorylating enzyme triokinase to glyceraldehyde phosphate. The two triose phosphate molecules then enter the E-M cycle in the direction of breakdown, or of synthesis to glucose-6-phosphate [154, 155]. In this way, large amounts of fructose are metabolized in the liver even in diabetes and independently of insulin. The liver is the site of fructose catabolism, while the metabolism of glucose takes place chiefly in the peripheral tissues. In contrast to the liver, fructose is metabolized very slowly and at least to some degree in relationship to insulin in the muscles and within the red cells [156, 157], so that in diabetes practically all oxidation of fructose takes place in the liver. In the muscles, fructose is either converted to fructose-6-phosphate and then directly into glucose-6-phosphate, or, to a lesser degree, fructose is converted to fructose-1-phosphate and then to fructose-1,6-diphosphate. Both pathways then enter the E-M cycle without an intermediate splitting of fructose to trioses, as occurs in the liver [153].

Sorbitol can also be used in the therapy of diabetes as a substitute for carbohydrates, as was shown in 1928 by Thannhauser. In mammalian liver, sorbitol undergoes loss of water to form fructose and is utilized as such by the diabetic [153, 158, 159].

Disturbances of Endocrine Regulation of Intermediary Metabolism in Diabetes

The individual reactions of the intermediary metabolism of the carbohydrates, fats, and proteins in the multicellular organism are not subject merely to the laws of mass action and the kinetics and thermodynamics of the catalyzing enzymes involved. These physical laws can guarantee the normal progress of the chains and cycles of reactions in intermediary metabolism only as long as the events of life are concentrated within a single type of cell. As soon as tissue differentiation and, concomitantly, differences in metabolism have developed, a system becomes necessary to coordinate the various separated metabolic processes to develop the optimal energy of the entire organ or tissue. The integration of the metabolic processes in different tissues at some distance from each other is under the control of the endocrine organs, which first occur in phylogeny at the same time as the first differentiation of tissues. In the diabetic, there must be a quantitative alteration in the activity or production of at least

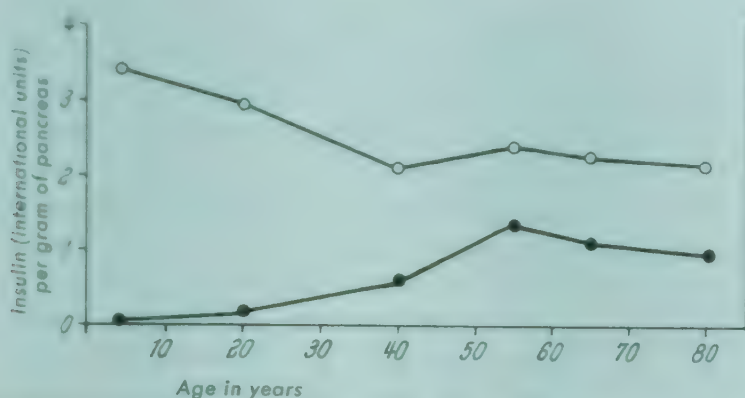


Fig. 58. The insulin content of the pancreas as related to age in normal (o) and diabetic (●) persons.

one hormone, which may be either the cause or the result of the diabetic disturbance of metabolism. As is well known today, both conditions are present: i. e., hypo- and hyper functions of endocrine organs can both lead to the development of diabetes, and also result from diabetes. It thus becomes ever clearer that there is no single endocrine defect to explain the various phenomena found in diabetes.

Normal carbohydrate metabolism demands of the body a complicated system of balances involving various compensatory and recoupling mechanisms, which homeostatically adjusts the carbohydrate content of the body to the existing body needs and produces optimal results. The control system is based on the unified action of many different hormones, none of which acts independently of the others. Any abnormal increase or decrease of each individual hormone can disrupt the regulating mechanism and result in a disturbance of the carbohydrate content of the body. Conversely, each such disturbance must lead to compensatory changes of the over-regulated endocrine control, with changes in the output and effect of various hormones. Insulin, of course, is of especial importance in this regard.

Physiology of Insulin

The discovery of the therapeutic activity of the hormone of Langerhan's islands in human diabetes by Banting and Best (1921) placed insulin deficiency in the foreground of the etiology of diabetes and for years determined the direction of clinical and experimental studies of diabetes. In addition, the pancreatic diabetes of Minkowski was produced by extirpation of the pancreas (1889), and alloxan diabetes of Dunn, Sheehan, and McLetchie was produced by a disturbed production of insulin by the B-cells of the pancreas (1943). The insulin deficiency theory was later modified because of the finding that hypophysectomy has a beneficial effect on diabetes, but a deficiency of insulin has remained the basic abnormality in this disease, to be finally confirmed by recent isotope investigations. An understanding of the role of insulin deficiency requires two prerequisites: understanding of the physiological effect of insulin and of the possible causes for its lack of action in diabetes.

The chief manifestation of the action of insulin, hypoglycemia, is the result of a number of parallel events which affect glucose. Together, these events involve the utilization of glucose and proceed at an increased rate per unit time under the action of insulin. This *increased utilization of glucose by insulin* has been shown in the intact animal [160, 161], in isolated tissues [162, 164], and in tissue slices [113, 188]. It is present both in the liver and also in the extrahepatic tissues, especially the muscles.

In the intact animal, it is expressed as a marked diminution of the extracellular glucose pool and of the glucose "turnover time," with no change in the "glucose space" (i. e., the space in the body through which the glucose distributes itself). Contrary to the opinions of Hastings [165, 189], the liver is directly involved at the beginning of the rise of glucose uptake and fat synthesis after insulin [160, 166]. However, it is doubtful if the uptake of glucose by the liver after insulin is greater than in the periphery [167], for the output of glucose by the liver is reduced on the one hand, so that an excess of glucose can be deposited in the liver; and, on the other hand, the liver exerts a stimulating influence on the peripheral uptake of glucose, which is mediated by insulin, and which may be misinterpreted in experiments on the intact animal as hepatic uptake of glucose. Following hepatectomy in the dog and the cat, the insulin-induced increase in glucose utilization in the isolated extremity and in the brain is less than in the intact animal [168, 169]. This observation indicates a hormonal influence of the liver on the action of insulin and agrees with the finding that the liver exerts a controlling action on carbohydrate metabolism in the sense of a homeostatic regulation of the peak level of blood sugar [170]. The liver thus assumes a central role in the metabolism of diabetes.

Best and his coworkers showed that the increased utilization of glucose caused by insulin is a complex phenomenon [160]. This could be divided into several partial processes, whose quantitative aspects were first satisfactorily studied 25 years later by means of isotopes (Table 50). First there is an *increased oxidation of glucose*, demonstrated both in the intact animal and in the eviscerated animal, as well as in tissue slices of muscle and liver [161, 163] (Table 51). In the individual tissue, however, the rate of oxidation apparently follows varying laws, since the increase can be more rapidly noted in extrahepatic tissues than in the liver itself. The conclusion that insulin generally has no direct effect on the liver was made by Hastings [165], but this contradicts the well-known preferential binding of insulin by the liver [172, 173, 174], as well as the observation that insulin stops the output of glucose from the liver within a matter of minutes [175, 176]. The delayed hepatic effect is probably related to the low hexokinase activity of the liver [177].

An *increased synthesis of glycogen* in the extrahepatic tissues parallels the increased oxidation of glucose [163, 178, 179]; this synthesis is maximal (in muscle) within minutes [165]. In the liver of a non-diabetic, on the other hand, insulin does not at first produce a change in the glycogen content. Following the compensatory tendency to glycogenolysis produced by hypoglycemia, there is a reduction of the glycogen content [178, 180, 181, 182]. In the patient with pancreatic diabetes, in contrast, and in starvation [72, 185], the synthesis of hepatic glycogen is greatly increased by insulin [183, 184]. Similar results are found in vitro provided that the liver slices are suspended in a potassium-rich "intracellular" medium, which is essential for the utilization of glucose by the liver cell [187]. Under these in vitro conditions, insulin induces the synthesis of glycogen from glucose only when the liver slices are taken from diabetic animals [188]. The cause for the failure of insulin to affect the glycogen content of the non-diabetic liver apparently is the fact that the non-diabetic liver already has a sufficient amount of insulin to produce maximal glycogen synthesis; i. e., an excess of insulin has no effect.

Insulin also decreases the output of glucose by the normal liver, and even more so by the diabetic liver [175, 176, 673]. This action is related to the increased utilization of glucose discussed above, and has the direct effect of reducing the level of circulating glucose. This action is at least partly explained by the effect of insulin in changing

the turnover of carbohydrates in the liver from synthesis of glucose to increased breakdown of glucose with concomitant synthesis of fatty acids. However, in addition, another specific action of insulin seems to be present: inhibition of the enzyme glucose 6-phosphatase [117, 189, 190], perhaps indirectly by diminished effect of hormones, such as cortisone, which are insulin antagonists.

The increased depletion of glucose in the isolated muscle and in the intact animal is not accompanied by any increase in oxygen utilization [162, 163, 171], and the newly synthesized glycogen comprises only a small portion of the diminished glucose. Hence, there can be no doubt that the increased glucose turnover following the administration of insulin must proceed by routes other than the synthesis of glycogen and the oxidation of glucose, and actually by a reducing process which counteracts an increased need for oxygen. The result is that *insulin causes an increased synthesis of fat from carbohydrate*; only 3% of the dietary carbohydrate is converted to glycogen, while 30% is converted to fat [186, 191-193]. This lipogenic action of insulin has two components: (1) there is increased conversion of glucose to fat as part of a generalized increase in glucose metabolism [194] because of increased passage of glucose into the body cells; and (2) there is an increased formation of fatty acids from the end products of the glycolytic catabolism of glucose (pyruvate, lactate, acetate) in the liver both in vivo [65, 195] and in vitro [196, 197], showing that insulin also acts on "active acetate" [70, 198]. In this case, there is a true activation of an energy-requiring process which can only proceed when the required energy is provided by parallel oxidation of glucose. Hence, the synthesis of fatty acids from acetate, lactate, and pyruvate is increased by insulin only if the liver already has a high glycogen content (because of prior feeding of a high-carbohydrate diet) [199]. In vitro, this occurs only if the nutritive medium contains both insulin and glucose. Isotope studies have shown that the increased synthesis of fat induced by insulin is the result of two effects of insulin on carbohydrate metabolism. The intensification of the D-H cycle, by producing TPNH, provides conditions for the synthesis of C₂ moieties (derived from the increased metabolism of glucose by way of the E-M pathway) to fatty acids [674]. The production of TPNH satisfies one of the most important prerequisites that fatty acid metabolism proceed in the direction of synthesis, for the hydrogen necessary for the reduction of β -unsaturated acyl-coenzyme A derivatives must be present in the form of reduced TPN [675, 676, 677].

Synthetic processes involving peptides and proteins are also involved in the insulin-induced depletion of glucose in the liver and other tissues. Insulin reduces the amino acid content of the blood of the normal animal from which the liver and kidneys have been removed [200, 201, 202] because the incorporation of amino acids into peptide-linkages (e. g., in glutathione, liver protein, muscle protein) is increased [112, 203, 204, 205]. At the same time, there is diminished excretion of amino acids and nitrogen in the urine, so that insulin spares protein. The amino acids which are reduced in the blood as a result of this action of insulin have the same relative ratio to each other as the amino acids in the newly formed muscle protein [206], suggesting that insulin has a direct stimulating influence on the synthesis of muscle protein from the amino acids of the blood. Salter and Best [207] showed that this deposition of protein by insulin makes insulin a "growth hormone" which produces an increased weight of the growing organism and of individual tissues. Like the lipogenic action of insulin, these effects are the results of an energy utilizing reaction and must be maintained by the oxidation of glucose, so that they proceed best in glycogen-rich tissues or, in vitro, when glucose is added to the culture medium [203].

Insulin also affects the potassium and phosphorus of the body. Insulin causes a fall of the serum levels of potassium and phosphate [208, 209] by increasing the passage of potassium and phosphate ions into the cells [171, 210, 211]. The uptake of phosphate by the cells is proportional to the increase in utilization of glucose and is of the same order of magnitude [171], indicating a close "coupling" of glucose and phosphate metabolism under the control of insulin. The same is true for potassium, which passes into the cells to the same degree and at the same time as glucose and inorganic phosphate, and is stored within the cell proportionately to the amount of glycogen synthesis [212, 213].

All these actions of insulin can be more or less completely imitated by an increase of the level of glucose in the blood, the circulating body fluids, or the culture medium. This has been shown for the increased uptake of glucose by the tissues [163, 168, 179, 214], the increased oxidation of glucose [73, 215] and the increased synthesis of glycogen in the peripheral tissues [216-218] and in the liver [219-221], the increased conversion of carbohydrate to fat [222], the cessation of output of glucose by the liver [223], the increased synthesis of peptides and proteins from free amino acids [224, 225, 227], and the uptake of phosphate by the cells [228]. The fact that the action of insulin can be replaced by a high blood glucose itself indicates that diabetic hyperglycemia is not only a direct result of a deficiency of insulin but is at the same time a necessary prerequisite which guards against further metabolic disturbances - a sort of automatic check system. The functional equivalence of hyperglycemia and the action of insulin indicate that *all the effects of insulin may be only secondary results of some fundamental mechanism* which produces the same results as does a marked increase of the extracellular glucose; i. e., *the easy passage of glucose into the cells*. Levine and Park were unable to confirm an older theory [46, 47] that the actions of insulin were due to an activation of hexokinase, and instead were able to establish the theory outlined above [51, 52, 229]. Initially, they found that when galactose was infused into a hepatectomized animal, it passed into the intracellular space only in the presence of insulin [61 a, 230, 231]. They were then able to show that the distribution of glucose between the intracellular and extracellular spaces also depended on insulin [56-59, 232-235]. They showed that the passage of glucose through the cell membrane, which was dependent on insulin, is due to an "active" transfer mechanism which depends on the specific molecular structure of glucose or other sugars. This specific structure occurs only in those monosaccharides whose carbon atoms C-1 to C-3 have the same structure as glucose itself [232] (Fig. 59). Thus, among the hexoses, insulin effects the transfer of D-glucose and D-galactose, but not of D-fructose or of D-mannose. Although not all authors accept the specificity of the action of insulin and suggest that there are different mechanism of transfer in different species of animals, there can be no doubt that insulin selectively activates the entry of glucose and galactose into the tissue cells and that this action is the basis of most of the actions of insulin. The growth-promoting action of insulin is also mediated by this membrane effect: the acceleration of the rate of mitosis by insulin depends on the entry of glucose into the cells, while fructose has no influence on the insulin effect [236]. The result is that insulin causes glucose to remain within the cell, thus increasing the production of energy necessary for mitosis and division [237].

The exact mechanism of transfer is not understood. As discussed on page 200, the liver and the kidneys contain an enzyme, mutarotase, which converts the glucose which passes through the cell membrane under the influence of insulin, into an easily

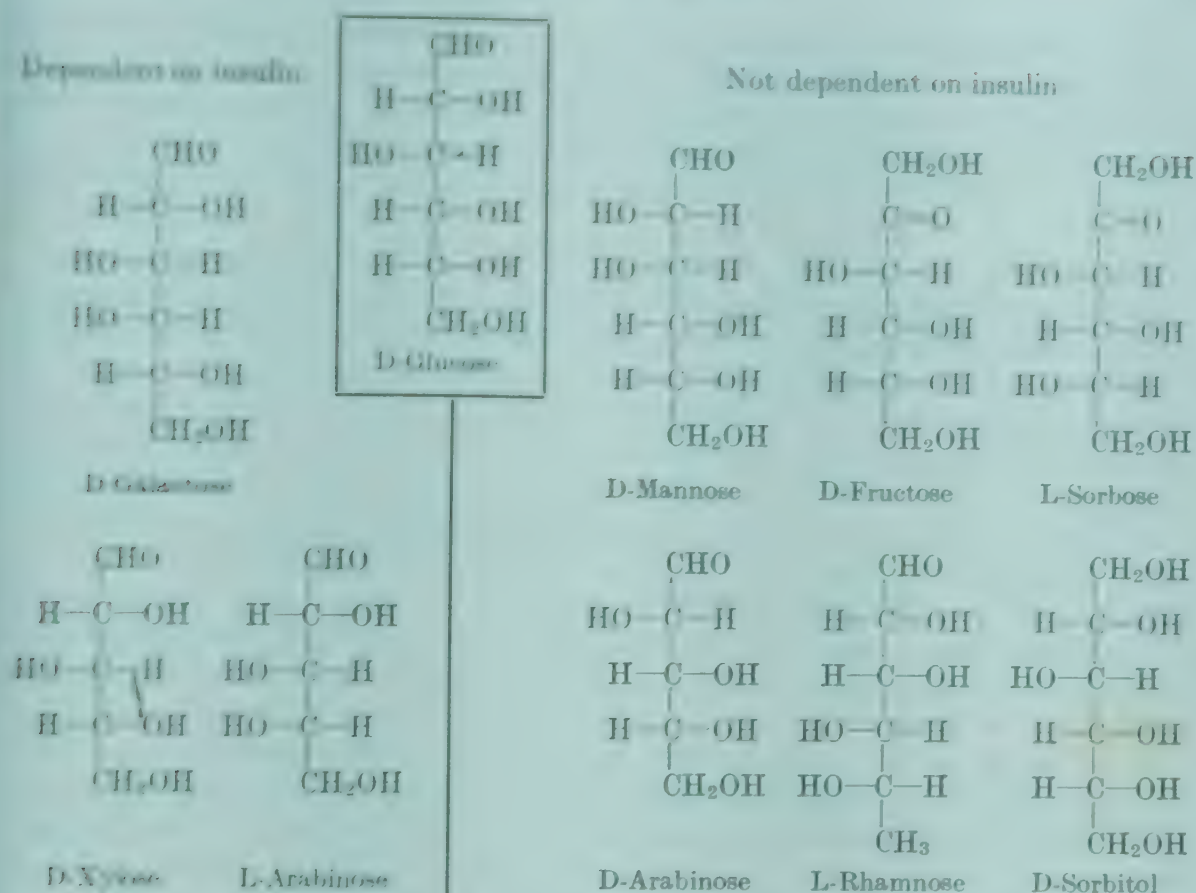


Fig. 59. The relationships between the chemical structure of the monosaccharides and the dependence of their metabolism on insulin. From Levine and Goldstein.

transportable form [60]. It may be that insulin takes part in the action of this enzyme. In any case, the mechanism which retains glucose within the cell, and which underlies most of the actions of insulin, is linked to an increase of oxidative phosphorylation [54, 238] with resulting increase of synthetic processes and an increase of the turnover of high-energy phosphate compounds (ATP and phosphocreatine) [45, 23-25, 228, 239-241]. The finding of normal P/O values in the liver mitochondria of rabbits with alloxan diabetes [242] does not negate the possibility of changes of the P/O values under *in vivo* condition [243].

The action of insulin is suppressed by anoxia [244, 245] and by poisons which inhibit oxidative phosphorylation, such as 2,4-dinitrophenol [246]. Insulin increases the ATP content of the liver and of red cells [23, 25, 241]. ATP, as is well known, is involved in the transport of phosphate through the cell membrane, which regularly accompanies the transport of glucose [171, 247-249]. It can therefore be assumed that the mechanism of glucose transport by insulin takes place as a result of increased turnover of high-energy compounds. This may occur only at the cell membrane, because insulin tends to combine with the surface of the cells whose glucose turnover it influences [248, 250]. This binding limits the site of action of insulin to the cell membrane or surrounding area. The tissue specificity of the insulin effect is not understood: the effect is limited to liver, cardiac muscle, skeletal muscle, mammary glands, and the lens of the eye. Insulin does not accelerate the transfer of glucose into the red cells, the intestinal tract, or the central nervous system.

The action of insulin in inhibiting glucose-6-phosphatase does not depend on the transfer of glucose across the cell membrane [251]. Here there is increased utilization of pyruvate and active acetate [62, 65, 95, 252, 254], which is due to activation of the turnover of high-energy compounds, and is impaired in the diabetic individual.

Orally Active Antidiabetic Agents

The most characteristic abnormality of the metabolic defect of diabetes is hyperglycemia. This is the expression of a profound, complex disturbance in the relationship between the production and the utilization of glucose, and is due in the last analysis to an absolute or relative deficiency of insulin. Adequate treatment of diabetes must therefore be based on the premise that it will return to normal those mechanisms which guarantee the homeostasis of blood glucose in health. When these mechanisms become normal, the production and utilization of glucose also become normal, and the energy requirements of the body are satisfied. Adequate treatment not only requires that the level of blood glucose approach normal, but also that, at the same time, the normal relationships among the individual metabolic pathways of carbohydrate, fat, and protein metabolism be reestablished. The hormone insulin most completely satisfies these requirements.

At the present time, there is no natural or synthetic substance which is capable of imitating the actions of insulin and thus substituting for insulin. However, there are many chemical compounds which are capable of lowering the elevated blood glucose of human and experimental diabetes. Study of the sites of action of such hypoglycemic agents in intermediary metabolism is of great importance in diabetic research.

The action of certain hypoglycemic compounds depends on a generalized or specific cytotoxic effect. Such agents are the guanidine derivative decamethylene diguanidine ("synthalin A") and related compounds such as the biguanides. In addition to their action in damaging general cellular metabolism (by way of accelerated glycolytic breakdown of glucose with simultaneous inhibition of the respiratory chain and corresponding deficit of energy [679], which is expressed as a fall in the glycogen content of the body [680, 681]), these substances have a special affinity for the glucagon-producing α cells of the islands of Langerhans. This cell system is so damaged morphologically and functionally that, in the case of synthalin, pancreatic glucagon falls to zero after administration of the drug. The hypoglycemic effect and the reduction of glycosuria are thus accomplished at the expense of severe generalized and localized cellular damage.

Many hypoglycemic compounds occur in plants [682]. Most of these have not yet been characterized as to their chemical nature or their mode of action in metabolism. Study of hypoglycin A [683, 684, 685, 686], an amino acid of structure β -methylene-cyclopropyl- α -aminopropionic acid [685, 686], has shown that these are compounds of unusual interest from the point of view of metabolic physiology. Hypoglycin A is found in the unripe fruit of *Blighia sapida* and is the substance responsible for the profound hypoglycemia which results from eating these fruits ("vomiting sickness" of the West Indies, West Africa, and Florida). The compound blocks oxidation of fatty acids, the oxidation of glucose is increased, and the ultimate result is hypoglycemia and decreased glycosuria in diabetic animals because of a diminution in the carbohydrate reserves of the body [687]. Studies of this compound have shown that the mere correction of diabetic signs in no way signifies biochemical correction of the disease.

In recent years, certain sulfonamide derivatives have become important in the treatment of diabetes. Since the advent of the aryl sulfonylurea drugs, several different theories have been put forward to explain their mode of action. Some of these theories are diametrically opposed to each other. Interpretation has been made especially difficult by virtue of the fact that different authors interpret the same findings in different ways.

Identical morphological changes in the B-cells after administration of these sulfonylurea drugs to animals have been interpreted by some as indicating increased function [696, 697, 703, 704], but by others as indicating functional arrest [691, 692, 693, 694]. In addition to the aryl sulfonyl butylurea compounds, sulfonamide compounds of thiazolol (IPTD) have shown similar hypoglycemic action [706, 707, 708], and many authors have assumed that results obtained for the one class of compounds also hold for the other class.

There are two fundamental experiments which must be discussed in any consideration of the action of the sulfonyl butylurea compounds. Firstly, various studies have shown unequivocally that these drugs are ineffective in the pancreatectomized animal [709, 710, 711, 712, 713], so that the endocrine function of the pancreas must mediate or limit the action of these drugs. Secondly, studies in animals with alloxan diabetes (i. e., after destruction of the insulin-producing B-cells of the islands of Langerhans) have shown that normal function of the B-cells is necessary for these drugs to be active. In the absence of the B-cells, administration of the sulfonylurea drugs produces neither hypoglycemia nor reduction of glycosuria [696, 697, 703, 714, 715, 716]. In contrast to these drugs, IPTD produces a reduction or even cessation of glycosuria in alloxan diabetes [706, 708, 717]. In those forms of diabetes in which functional B-cells are still present (e. g., in "idiosteroid" diabetes), the sulfonylurea compounds show hypoglycemia effects [696, 703, 714].

These findings demonstrate that the insulin-producing B-cells of the pancreas are necessary for the hypoglycemic effect of the aryl sulfonylurea compounds. They give no clue, however, as to whether insulin must merely be present, or whether the drugs directly cause an increased secretion of insulin. The morphologic changes in the B-cells [714, 697, 696, 699, 718, 719, 720] strongly suggest that there is increased activity of the B-cells after the administration of the aryl sulfonylurea compounds. The increased secretion of insulin has been shown by biochemical studies in parabiotic animals [698]. In addition, the insulin content of the pancreatic veins is increased after the administration of these drugs [721]. In agreement with this result is the finding that the injection of small, peripherally ineffective amounts of the sulfonylurea drugs into the pancreatic artery causes a fall of the blood sugar [722]. Blood drawn at the point of maximal hypoglycemia following the administration of either aminobenzol sulfonyl butylurea or toluyl sulfonyl butylurea shows increased amounts of insulin [723, 724, 725, 726], and at the same time the insulin content of the pancreas falls [727, 728].

The doses of the sulfonylurea compounds used in these animal experiments are relatively high as compared with those used therapeutically, and the experiments show increased secretion of insulin following such large doses. In diabetic persons, however, the methods for the determination of insulin are so inexact and insensitive that no increase in plasma insulin can be shown [729].

Despite the finding of increased secretion of insulin in animal experiments, the metabolic effects of the oral antidiabetic drugs cannot immediately be explained by the increased amounts of endogenous insulin. When insulin produces hypoglycemia in

a fasting animal, the glycogen content of the liver remains unchanged or falls and at the same time the concentration of glycogen in the muscles increases. The hypoglycemia induced by the aryl sulfonylurea drugs, however, is accompanied by an increase of the liver glycogen with no change in the concentration of muscle glycogen [696, 714, 701]. Hence, there must be an effect on the intermediary metabolic processes within the liver.

However, the fact that the increase in liver glycogen typical of the aryl sulfonylurea drugs may also occur in the absence of hypoglycemia [703] suggests that the increased liver glycogen cannot be considered an expression of a reduced output of glucose from the liver which in turn might be responsible for the hypoglycemia. In accord with this concept of a subordinate action of the liver as regards hypoglycemia is the finding that hypoglycemia is produced by the oral antidiabetic drugs even in the hepatectomized animal [701, 730]. One may conclude from these findings that there is increased uptake of glucose by extrahepatic tissues following the administration of these sulfonamide drugs to animals with intact islets of Langerhans. Studies with radioactive glucose confirm the fact that, despite the constant muscle glycogen, there is an increase in the amount of glucose turned over in the muscles [731, 732]. At the same time, however, there is increased transformation of glucose into liver glycogen [731, 732]. It can be shown in experimental animals that the slow infusion of insulin into the portal vein over a period of several hours causes increased incorporation of radioactive glucose into liver glycogen [733]; this finding is in contrast to that seen after the peripheral injection of insulin. In other words, insulin given via the portal vein has a similar action to that seen after the administration of the sulfonylurea derivatives. However, the action of these drugs in increasing the liver glycogen of fasting animals must be distinguished from the increased incorporation of glucose into liver glycogen, for prolonged administration of the sulfonylurea drugs causes an increase of the total liver glycogen but without increase of specific activity after administration of radioactive glucose [703, 732]. Under the same conditions, there is neither hypoglycemia nor increased secretion of insulin [723]. The sulfonylurea drugs no longer cause an increase in liver glycogen after adrenalectomy [701, 703], so that this effect must be mediated by the adrenal cortex. There is increased transformation of C^{14} -labelled glycine into glycogen [731], so that part of the increase in glycogen is the result of increased gluconeogenesis.

The increased glycogen of the liver is thus an expression of both increased uptake of glucose from the extracellular space and accelerated conversion of amino acids to glycogen. The finding that adrenalectomy results in increased sensitivity to the aryl sulfonylurea drugs [696, 712, 714] which is greater than that seen after hypophysectomy [712] suggests that the effect of these drugs on adrenal cortical function may be due to a direct action on the adrenal glands. This concept is not contradicted by the finding that there is no change in the total excretion of the end metabolic products of the corticosteroids after administration of the sulfonylurea drugs [734, 735], since it is conceivable that the total amount of corticosteroids may be unchanged and yet that there might be a change in the composition of the individual hormones which could lead to the observed elevation of liver glycogen. Studies were undertaken to determine the degree to which impaired breakdown of liver glycogen might contribute to the reduction of blood sugar in acute experiments, above and beyond the increased uptake of glucose by the peripheral tissues [736, 737]. These studies determined the rate of turnover of the extracellular glucose pool. In rats, it was found that the oral administration of aminobenzol sulfonyl butylurea was followed first by a rise in the

peripheral uptake of glucose, while the rate of output of glucose from the liver was unchanged. Thus, the initial fall of blood glucose is due to increased peripheral utilization of glucose. Later on, the output of glucose from the liver falls. The overall result is prolonged hypoglycemia with delayed turnover of blood glucose. The same results have been found in man after the administration of therapeutic doses of toluyl butyl urea [738].

The increased uptake of glucose from the blood together with the increased conversion of C^{14} labelled glucose into muscle and liver glycogen and the occurrence of hypoglycemia after the administration of the oral antidiabetic drugs to the hepatectomized animal can all be considered manifestations of a peripheral action of insulin. In agreement with this concept is the finding of increased glucose oxidation in gas metabolism experiments [702, 739]. In contradiction, chiefly because of different types of experiments, are the data concerning the influence of differences between arterial and venous levels of blood glucose [729, 740, 741, 742].

In addition to the changes in metabolism discussed above, the aryl sulfonylurea drugs also have direct inhibitory actions on certain enzyme processes [743, 744, 745, 746]. These *in vitro* effects, however, first become manifest at relatively high concentrations of the drug, and it has not yet been shown that these high concentrations can be reached within the body cell. Similar inhibitory effects also occur with sulfonamides which do not affect the blood glucose. Thus, for example, both the aryl sulfonylurea drugs [743, 744, 745] and gantrisin [745] inhibit the reactivation of liver phosphorylase, which occupies a key position in glycogenolysis. *In vitro*, it is possible to show inhibition of hepatic glucose-6-phosphatase in the presence of high concentration of aryl sulfonylurea compounds [747], but this cannot be demonstrated at the point of maximal hypoglycemia in animals treated with these drugs [696, 714, 747], so that this inhibition cannot play a part in the development of the acute hypoglycemia. However, prolonged treatment with the oral antidiabetic drugs leads to demonstrable reduction of the activity of this enzyme within the liver [741, 748]. This effect is due to increased insulin activity, for insulin itself has the same effect on this enzyme [749].

It has also been shown that the aryl sulfonylurea compounds have the property of acting both as competitive inhibitors and as activators in certain enzyme reactions which depend on pyridine nucleotides [750]. These effects may be used to explain the different levels of, for example, pyruvate after the administration of insulin as opposed to aryl sulfonylurea [750, 751, 752, 753]. Considering the known fact that there is no fall in blood glucose or decrease of glycosuria in alloxan diabetic or pancreatectomized animals, the changes of glucose metabolism due to these actions obviously are inadequate to bring about a fall in blood glucose in the absence of insulin. It is possible, however, that a hypoglycemia which is already present may be indirectly potentiated in this way [750].

In addition, certain other actions of the oral antidiabetic drugs require the presence of insulin but are not dependent on the presence of functional B-cells in the pancreas. These drugs thus potentiate the action of suboptimal doses of insulin in pancreatectomized animals [754, 755, 756] and in animals with alloxan diabetes [703]. This potentiation first appears after prolonged treatment with insulin. As yet, it is not known whether this effect can be explained by inhibition of insulin breakdown [757], as found *in vitro* under the influence of the sulfonamides [695] or is the consequence of a resynthesis of limiting enzymes which are produced only after prolonged insulin therapy.

A limiting factor for the development of the hypoglycemic action of the "antidiabetic urea compounds" is the capacity of the insulin-producing B-cells [703, 704].

Animal experiments have shown the development of increasing resistance to the hypoglycemic effects of the aryl sulfonylurea compounds following prolonged administration of these compounds [703]. At the same time, no additional secretion of insulin can be detected, as measured by the insulin activity of the plasma [723] and increased synthesis of muscle glycogen [732]. Such animals show decreased glucose tolerance [703]. These changes are even more marked in animals in which the B-cell system has been reduced prior to the prolonged administration of the aryl sulfonylurea compounds, as by the administration of subdiabetogenic doses of alloxan [758] or by partial pancreatectomy [759]. In experiments with forced feeding of a carbohydrate diet, the disturbed utilization of glucose becomes evident and marked glycosuria occurs [737]. At the same time, the turnover of blood glucose is delayed [736]. These changes which occur under extreme conditions in the experimental animal appear as the logical result of diminished adaptability of the B-cells which have either been overstimulated or have become incapable of storing insulin.

In summary, it can be stated that the aryl sulfonylurea compounds cause increased secretion of endogenous insulin, but that they themselves do not have insulin-like action. The metabolic effects of the endogenous insulin, however, are overlapped by more or less nonspecific effects of the sulfonamides on enzyme systems and endocrine glands, so that there is partial masking of the insulin action.

Deficiency of Insulin in the Etiology of Diabetes

The fundamental findings in diabetes are the result of pathological diminution of the action of insulin, and can be more or less completely corrected by the administration of insulin. This holds for the following abnormalities of human and experimental diabetes:

- (1) Diminished uptake of glucose by the cells [120, 163, 255].
- (2) Diminished oxidation of glucose [120, 194, 255].
- (3) Diminished synthesis of glycogen in the liver and other tissues [120, 122, 182].
- (4) Increased output of glucose from the liver, with increased blood glucose [120, 175].
- (5) Diminished lipogenesis [194].
- (6) Diminished synthesis of peptides and proteins [112].
- (7) Diminished utilization of pyruvate and active acetate [70, 254].
- (8) Diminished synthesis of high-energy compounds [254].

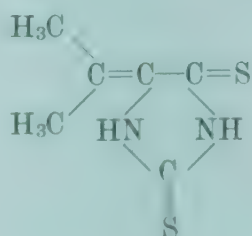
The diminished insulin activity in diabetes may be due directly to a deficiency of insulin – i. e., *insulin-deficiency* diabetes. In such cases, the parenteral administration of insulin can restore normal metabolism. In other cases, however, the insulin deficiency may be due to a different mechanism, in which the physiological effect of insulin is counteracted by insulin antagonists. Such cases of diabetes do not respond to the administration of insulin but are *insulin-resistant*.

Insulin-deficiency Diabetes. The first type of diabetes – “insulin-sensitive,” “labile,” or “insulin-deficiency” diabetes – is seen chiefly in children and in thin individuals. It may be due to many different causes. Most commonly there is a functional diminution of the insulin-producing B-cells of the pancreas, with or without morphologic changes. The insulin content of islet tissue is in general lower in childhood than at later ages, and rises in proportion to the logarithm of the age in days [256, 257], with simultaneous increase in the ratio of the number of B-cells to A-cells. Conversely, the total insulin content of the pancreas is reduced with increasing age, because the exocrine portion of the gland grows more rapidly than the endocrine portion. The insulin content of the pancreas in diabetes is significantly reduced, to 3–5% of the normal in juvenile diabetes (up to 20 years of age), and to 50–60% of the normal in elderly

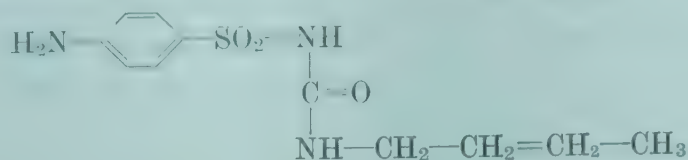
patients (50 years and older) (Fig. 58). In juvenile diabetes with extreme depletion of insulin, the B-cells of the pancreas are markedly atrophic, while they are not demonstrably reduced in number in adult diabetes. Hence, it is only juvenile diabetes (so-called "growth-onset diabetes") which is a pure type of insulin deficiency, while in adult diabetes those symptoms which are due to insulin deficiency are largely governed by counter-regulatory hormones which are anti-insulin in their actions (p. 223).

Usually, in juvenile diabetes, the diminished production of insulin is the direct result of atrophy of the B-cells. In addition, however, insulin deficiency may also result indirectly as a result of increased destruction of insulin within the body. Normally, the insulin which is taken up by the tissues and is bound by a specific binding is catabolized by specific cellular enzymes, insulinases, which are found chiefly in leukocytes, liver, and the kidneys [258-261]. The insulinase activity of the liver is especially marked [259, 262], although it varies under varying conditions of nutrition, being elevated when the diet is rich in carbohydrates and reduced in starvation [263, 264]. Hepatic insulinase is a protease with an optimum pH of 7.6 to 8.0 [265]. Its high activity is of interest because insulin itself is preferentially bound by and stored in liver cells [172-174]. In addition, there is increased insulinase activity in human diabetes [267 b, 267 c]. The reduced insulin requirement of diabetics with liver and kidney disorders, and the tendency of patients with hepatic disease to have hypoglycemia and reduced blood amino nitrogen, are probably at least in part related to a diminution of the insulinases in these tissues [266]. The insulinase activity of the liver is under the control of the anterior pituitary and is markedly reduced by hypophysectomy; this fact partly explains the hypersensitivity to insulin which occurs in pituitary insufficiency [261]. The activation of insulinases by the anterior pituitary is not mediated by the adrenal glands [261] and cannot therefore be attributed to ACTH. Hence, there is a connection between true insulin-deficiency diabetes and the "counter-regulatory" diabetes of older individuals ("maturity-onset diabetes"), which is caused by anti-insulin hormones. In the latter, therefore, there is also a component of insulin deficiency which may be the result of increased activity of insulinases.

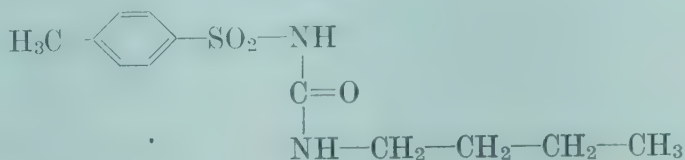
Thyroidectomy also reduces insulinase activity [261]. It is still uncertain whether the activation of insulinases by the anterior pituitary hormones [261] and by thyroxine [261] is a direct action or is due to removal of an inhibitory substance. The liver contains an insulinase inhibitor which is peptide in nature and which inhibits the enzyme competitively and reversibly [267 a]. In itself it has no action on the blood sugar, but it has a marked potentiating action on subsequently injected insulin in both rabbits and rats [267 a]. The striking hypoglycemic action of L-tryptophane, indol-3-acetic acid, and nicotinic acid is similarly due to the fact that these substances are competitive inhibitors of insulinases [267 b, 267 d]. In addition, there are non-competitive inhibitors which can inactivate or destroy insulinases chemically, such as all the SH-binding compounds (Cu-, Zn-, Hg-salts; iodoacetic acid). Another such inhibitor is 5-isopropylidene-2,4-dithiohydantoin [261]:



Others are certain arylsulfonylurea derivatives such as Nadisan (Carbutamide)



and D 860 (Rastinon) (Tolbutamide, Orinase)

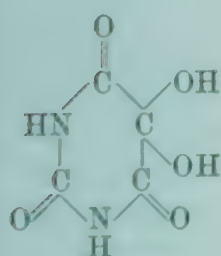


These compounds, which in vitro are non-competitive inhibitors of insulinase [267 c], have recently assumed therapeutic importance as oral antidiabetic compounds. The anti-insulinase activity of the arylsulfonylureas may explain why these substances have no effect on the blood sugar level in alloxan diabetes, where no insulin is produced [398, 399], although they potentiate the effect of insulin administered to animals with alloxan diabetes [267 e]. In the foreground of the action of these arylsulfonylurea derivatives there is an increase of insulin production [267 e], so that there are two ways in which there is intensification of insulin action. In apparent contradiction to the postulated increase of insulinase activity in diabetes is the observation that injected radioactive insulin disappears more slowly from the blood of diabetics than of normals, and is more slowly broken down in the diabetic than in the normal person [268]. This observation, which is yet to be confirmed, perhaps indicates that the impaired binding of exogenous insulin to the cells in patients with diabetes [172-174, 218, 250] constitutes the prerequisite both for its destruction and its activity at the cell surfaces.

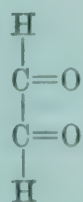
Lack of insulin action may also occur, at least therapeutically, as a result of antigenic activity of insulin manufactured from other animal species, so that antibody production neutralizes the insulin administered. In the guinea pig and the sheep, heterologous antigenic insulins can produce anaphylactoid or allergic reactions [269]. Allergy to insulin and the formation of anti-insulin antibodies are events which run parallel to each other but which can be distinguished from each other [270, 271]. The formation of antibodies to heterospecific insulins produces insulin-resistance in animals [272]. The injection into mice of guinea-pig-insulin-immune-serum causes severe diabetes with acetonuria [269]. Patients with diabetes can also acquire resistance to insulin as a result of the formation of antibodies to heterologous insulin [270, 271, 273, 274]. On the other hand, human diabetes due to autoantibodies does not occur, because endogenous insulin does not stimulate the formation of antibodies. The insulin resistance which develops in patients with diabetes develops against some, but not all, heterologous insulins, and the antibodies which appear in the serum of such diabetics also react with biologically inactive insulins which have been inactivated with cysteine. Thus, the biological activity and the antigenicity of insulin have different chemical specificities [274]. The insulin antibodies of human serum, which can be demonstrated by a variety of techniques (Coombs test, complement fixation, hemagglutination), show marked fluctuations in the same individual and can disappear just as suddenly as they appeared [275]. This fact is probably the explanation for the occurrence of transient resistance to insulin during infections.

Alloxan Diabetes. In animal experiments, pure insulin deficiency diabetes can be produced only by direct extirpation of the insulin-producing B-cells of the pancreatic islets. The classical diabetic model of von Mering and Minkowski in 1889 does not fulfil this ideal because extirpation of the pancreas removes not only the B-cells but also the A cells, which produce the insulin-antagonist "glucagon." The resultant pancreatic diabetes thus differs from spontaneous human diabetes in the fact that the A-cells are also missing, together with their hormone. The metabolism of human diabetes is more closely approximated by another form of experimental diabetes, *alloxan diabetes* (Dunn, Sheehan, McLetchie 1943). Alloxan has a selective destructive action on the B-cells in all mammals except guinea pigs, without affecting the A-cells and the rest of the parenchyma of the pancreas [276, 277]. Parenterally administered alloxan first produces a marked hypoglycemia as the result of acute stimulation of the B-cells, which is rapidly followed by degranulation, hydropic degeneration, necrosis, and atrophy of the B-cells. The result is the development of a permanent diabetes. Alloxan diabetes, like human diabetes, also exhibits pituitary and adrenal components (p. 200), so that alloxan diabetes is an excellent experimental model for human diabetes.

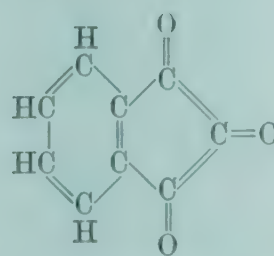
The exact mode of action of alloxan is not known. Alloxan reacts within the islet tissue both with the SH-groups of the mercapto enzymes and of glutathione [103], and with amino acids, which undergo decarboxylation and deamination. Alloxan also reacts with the trace element zinc, which apparently mediates the fixation of insulin to the protoplasm of the B-cells [278]. All three actions are involved in the resulting damage to the B-cells. The dicarbonyl group of alloxan is necessary for its diabetogenic action, for a number of other compounds with two or three carbonyl groups also cause destruction of the B-cells and are therefore diabetogenic. Most of these compounds are cyclic. Such compounds include glyoxal [279], isatin [280], uric acid [281], ninhydrin [282], and possibly barbituric acid [283, 285] and dehydroascorbic acid [101, 284], together with various derivatives of these substances [285]. The diabetogenic action of these substances is weaker than that of alloxan and often requires additional prerequisites (for example, uric acid requires a sulfur-poor diet). Many of



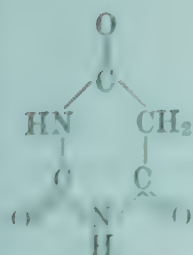
Alloxan



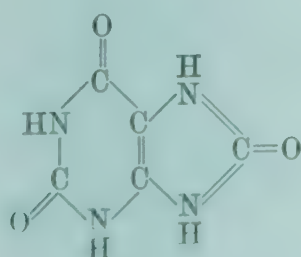
Glyoxal



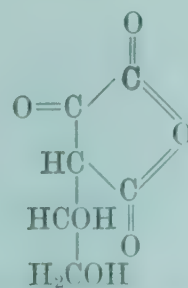
Ninhydrin



Barbituric Acid



Uric Acid

Dehydroascorbic
Acid

these substances are found in the body or are often taken in food, so that this mechanism for the production of diabetes may be of some importance in human diabetes [286-288].

The ability to react with zinc and thus prevent its physiological action of binding insulin to specific proteins of the B-cells is a property which alloxan shares with other substances which thus have a similar diabetogenic action. Diphenylthiocarbazone, 8-hydroxyquinoline, and their methyl derivatives are compounds which form such zinc complexes and thus produce a permanent insulin-deficiency diabetes with destruction of the B-cells. At the same time, they cause a marked fall of the zinc of the islets, which is excreted in the urine [289-292]. The removal of zinc by such substances prevents the binding of insulin to the islet substance, a binding which is necessary for the functional and morphological integrity of the B-cells. On the other hand there are non-toxic substances which build complexes with zinc, and these either have no diabetogenic action at all, or actually prevent the development of diabetes [292]. The significance of the binding of zinc in the development of diabetes is not completely understood [293].

Blood Glucose. Of great importance in the study of diabetes is the fact that the production and output of insulin by the pancreas is governed by the level of the blood sugar. The homeostatic control of insulin activity by the blood sugar is so marked that glucose has been called the "hormone of insulin output" [294]. The injection of glucose into the pancreaticoduodenal artery does not cause an elevation of blood glucose but rather hypoglycemia in the peripheral blood or in a parabiotic animal [294-297]. Prolonged infusion of glucose into the hepatic or gastroduodenal arteries of the dog produces a marked and permanent hypoglycemia, which can be maintained for as long as 18 days [298]. The injection of glucose into the portal vein has no such action, so that the effect is not due to any action of the liver. The effects must therefore be due to an increased output of insulin due to an elevation of the blood glucose in the blood supply to the pancreatic islets [295-297]. At the same time hyperglycemia causes hyperplasia of these islets [299]. In the isolated pancreas and in pancreatic tissue slices, the production of insulin is increased when glucose is added to the perfusion fluid [300, 301]. The blood plasma of normal persons shows an increased content of insulin after the oral administration of an oral dose of glucose, while no insulin appears in the blood of insulin-deficiency diabetics after oral glucose [302]. Older diabetics with a tendency to insulin-resistance show normal amounts of plasma insulin following oral glucose, indicating that they are not deficient in insulin [303]. A high-carbohydrate diet similarly increases the insulin content of the pancreas [304]. Conversely, prolonged starvation and a carbohydrate-poor diet cause a reduction of the insulin content of the pancreas which may approach zero, with accompanying inactivity of the islet cells [305, 306]. When prolonged hypoglycemia is produced by sufficiently long administration of exogenous insulin, the islet cells show degranulation [307] and the production of insulin by the pancreas is so diminished that true diabetes with hyperglycemia and glycosuria develops - so-called "insulin diabetes" [308, 309]. Nothmann showed in 1931 that the injection of a large dose of insulin suppresses the production of insulin by the pancreatic islets for several hours [310]. The blocking of the output of insulin by the pancreas in prolonged hypoglycemia, together with the diminution of adaptive enzymes of glucose oxidation, is a fundamental cause of "hunger diabetes" (p. 190).

In certain species of animals, when the elevation of blood glucose in the above experiments is maintained over a long period of time, the resulting stimulation of insulin

production can place so great a demand on the B-cells of the pancreas that cellular exhaustion occurs (degranulation, vacuolization, hyaline degeneration, necrosis). This may be seen in cats [311], hogs [312], and under certain conditions in dogs [298], rats [313], and guinea pigs [314]. The result may be the development of transient glycosuria or even, when the islet cells are overloaded for some time, of a permanent diabetes ("hyperglycemic diabetes") [311]. This kind of B-cell exhaustion following prolonged elevation of the blood sugar can also be produced by overfeeding of carbohydrates [315], by partial pancreatectomy [316], and by prolonged administration of "diabetogenic" hormones.

Partial pancreatectomy in such experimental animals is followed by an exaggerated response of the remaining islet tissue, and the resulting diabetes is called "Sandmeyer diabetes."

In 1937, Young had already observed that the parenteral injection of anterior pituitary (hyperglycemic) extracts, which at first produces only a transient glycosuria ("idiopathophysical" diabetes), later, on repeated injection over a period of many days, results in a permanent type of diabetes which persists after the injections are stopped ("metahypophysical" diabetes) [317]. The constant hyperglycemia produced by the anterior pituitary hormones has the same effect on the B-cells as the injection of glucose itself: the B-cells are exhausted, changes of degeneration appear, and the insulin content of the cells is reduced, so that a permanent diabetes follows [318-320]. The pancreatic origin of pituitary diabetes is also shown by improvement of this diabetes by the administration of insulin [321]. The same "metahypophysical" diabetes is seen following the repeated use of the pure diabetogenic hormone of the anterior pituitary, the growth hormone in dogs and cats [322, 323], and the corticotrophic hormone in rats and rabbits [324]. In all these cases, stimulation of the insulin production of the pancreas precedes the degenerative changes of the B-cells which lead to diabetes [325]. An analogous "metacortical" insulin-deficiency diabetes develops in partially pancreatectomized dogs [326] and rabbits [327] following the injection of cortisone. An analogous "metathyroidal" insulin-deficiency diabetes similarly occurs in partially pancreatectomized dogs after prolonged administration of thyroxine or triiodothyronine [328]. When there is pathologically increased production of the corticosteroids, as in Cushing's syndrome, the insulin content of the pancreas falls to one-fourth of the normal [329]. In the dog, a peculiar form of diabetes has been produced by repeated injection of the lactotropic hormone of the anterior pituitary gland ("metaprolactin" diabetes) [330]. It is not known whether this observation has any relationship to the development of diabetes in women at the termination of pregnancy.

All these "metahormonal" types of diabetes are alike in that they are due to a deficiency of insulin and must be distinguished from the direct results of the action of diabetogenic hormones. These forms of diabetes, which are the direct result of chronic hyperglycemia—hyperglycemic diabetes, Sandmeyer diabetes, metahormonal diabetes—have been seen *only* experimentally. Many recent observations in man have shown that persistent hyperglycemia, even if present for as long as several decades, does not lead to irreversible damage of the islet cells [331-333] or to reduction of the insulin content of the pancreas [256]. Thus, a mild diabetes with moderate hyperglycemia does not generally tend to become worse. The results of the animal experiments with posthyperglycemic diabetes thus cannot be applied to man. Thus, in the treatment of severe cases of diabetes, small degrees of persistent hyperglycemia can be accepted without worry as against the greater risk of overdosage with large amounts of insulin.

The complex system of hormonal control indicates that insulin is formed within a complicated system of homeostatic and compensatory devices which result in an optimal amount and activity of insulin. It is important to understand the role of the other hormones which control insulin activity, since a disruption of the regulating mechanism or a dissociation between the action of insulin and the counteraction of other hormones may result in the development of diabetes-like states.

The counter-regulatory processes stimulated by the action of insulin are multiple, an indication of the importance to the organism of the maintenance of a constant blood sugar level and of the adjustment of changes of carbohydrate metabolism. Compensatory mechanisms are set in motion, usually, by a fall of the blood glucose and not by insulin itself, although it is not always possible to distinguish between the two possibilities. The counterregulatory mechanisms which result from an outpouring of insulin or a reduction of blood glucose below normal levels are so intense, that they result in a "self-limiting effect" of insulin itself (Somogyi). This is shown by the fact that the action of injected insulin is shorter and more transient, the more severe the initial hypoglycemia. In addition, the same dose of insulin which causes a reduction of blood sugar in the presence of hyperglycemia causes an elevation of blood sugar when the level is normal or low [334]. There is always the possibility that exaggeration of the self-limiting effect because of pathological responsiveness of the counterregulatory mechanism may also lead to diabetes, as occurs when there is an abnormal increase in the production of insulin over a long period of time (p. 222).

The nature of these humoral factors which govern the action of insulin is extraordinarily complex. The maintenance and restoration of a certain minimal blood glucose level is a basic essential of life, which must be guaranteed by a number of parallel mechanisms. Involved in these processes are the hormone of the A-cells of the pancreas, glucagon; epinephrine; ACTH of the anterior pituitary gland; and the glucosteroids of the adrenal cortex. All these hormones have an anti-insulin action. In addition, the liver also influences the action of insulin in a manner which is as yet unknown. Insulin increases the glucagon content of the pancreatic islets [335] and causes a pouring out of glucagon from the A-cells [336]; the curve of blood sugar following a load test is determined by the interacting role of these actions [336, 337]. If insulin causes a fall of the blood sugar to abnormally low levels, there is an immediate outpouring of epinephrine [334, 338-341] in an amount which is proportional to the degree of hypoglycemia [342]. At the same time, the administration of insulin increases the amount of epinephrine in the urine [343]. Extirpation of the adrenal medulla increases insulin hypoglycemia [347]. Glucagon and epinephrine are thus direct, injurious antagonists to insulin.

In addition, epinephrine also has an indirect anti-insulin action which is mediated by the anterior pituitary and adrenal cortex (p. 229). However, both the anterior pituitary and the adrenal cortex also respond directly to insulin. Thus, insulin causes a secretion of ACTH which is independent of epinephrine [344], increasing the blood concentration of ACTH [346]. Insulin prevents the degranulation and vacuolization of the ACTH-producing basophilic cells of the anterior pituitary which are seen following pancreatectomy [345]. Insulin also stimulates the activity of the adrenal cortex, an action which is mediated by ACTH and is independent of epinephrine. The resultant increase of corticosteroids counteracts the action of insulin [347-350]. This effect is due to the hypoglycemia and not to insulin itself [347, 348]. The effect wears off after the counter-regulatory synthesis of glucose is finished and leads secondarily to cessation of adrenal cortical activity [351]. Insulin thus triggers a complex homeo-

static mechanism in the adrenal cortex which ultimately leads to suppression of the function of the cortex after normoglycemic levels are reestablished. In diabetes, regulatory mechanisms are faulty or lacking. In an analogous two-phase regulation in diabetes, hyperglycemic states first cause reduction of hormone synthesis in the adrenal cortex and then produce adrenal cortical hyperactivity as a reaction to stress, with aggravation of the diabetes (p. 231) [352 to 354]. The degeneration of the corticosteroid producing zona fasciculata following pancreatectomy is apparently the result of prolonged functional overactivity of the adrenal cortex [355].

The homeostatic functions of the *liver* as regards the control of the actions of insulin were first investigated by Soskin and his group, who suggested that they were important in the maintenance of the level of blood glucose. To date, it has not been possible to identify these factors, which work partly on carbohydrate metabolism and partly on insulin. Hormone-like liver substances increase the action of insulin in the peripheral tissues (p. 210), and the action of insulin seems to depend on the presence of normal hepatic function. The latter fact is demonstrated by the observations that glucose tolerance tests in hepatectomized animals with intact pancreas are diabetic in type, while those in insulin-treated pancreatectomized animals with intact liver are normal [356]. In addition, the hepatectomized animal shows severe disturbances in carbohydrate metabolism which are incompatible with life [357], while rats and rabbits whose pancreatic islets have been extirpated can have a normal blood sugar and show good general health for a long time (p. 227) [358]. The same is true of the *Houssay animal* (p. 235).

These hepatic observations indicate a phylogenetically old control by the liver of regulation of the blood sugar. Impairment of this relationship leads to the diabetic type of glucose tolerance curve, as seen in hepatic damage, and to the metabolic aberrations seen in pancreatic diabetes. The result is always related to liver damage because of the absence of lipotropic material of the pancreas, and insulin cannot correct the liver damage [359-361]. On the other side, anti-insulin substances include the insulinases of the liver (p. 219) and the lipoprotein inhibitors of hexokinase which are produced in the liver (p. 235). Diminution of these anti-insulin substances in parenchymatous liver disease can result in improvement of the carbohydrate tolerance and a reduction of the insulin requirement in diabetics (p. 235). The enzyme glucose-6-phosphatase, which is directly responsible for the formation of blood glucose, is produced only in the intact liver, and this enzyme is greatly reduced in liver disease [362]. The result is a fall of the blood glucose. In these respects, the liver, as an organ which produces insulin antagonists and substances inhibited by insulin, may be considered in the same category as the A-cells of the pancreas, the anterior pituitary, and the adrenal cortex. Damage to the liver can have different results depending on which processes in the liver are damaged: there may sometimes be impairment of the action of insulin, at other times potentiation of this action, with correspondingly unfavorable or favorable effects on diabetes.

The fact that insulin favors the synthesis of high-energy phosphate (p. 213) explains the effect of thiamin on diabetic metabolism, since the conversion of thiamin into its active form (thiamin pyrophosphate or cocarboxylase) can occur only if ATP is present. This conversion therefore depends indirectly on the presence of insulin [363]. Insulin deficiency therefore produces a deficiency of cocarboxylase in the body, and administration of this coenzyme seems to increase the action of insulin especially in patients with severe diabetes. Within the body, thiamin is fixed only as cocarboxylase and never in the free form, so that most thiamin is lost in the diabetic and

thiamin deficiency may occur in diabetes. The resulting manifestations appear chiefly at those stages of carbohydrate metabolism which depend on carboxylase, and these in turn must be intact in order for insulin to work. Insulin cannot work, therefore, without the presence of adequate amounts of thiamin (thiamin pyrophosphate) in the body [364]. When thiamin deficiency is present, glycosuria and other diabetic signs may develop even in the presence of insulin [365], and these can be eliminated by administration of thiamin.

Glucagon

As time goes on, it becomes more and more apparent that the hormone of the A-cells of the pancreatic islands plays a basic, although as yet incompletely understood, role in the etiology of diabetes. This hormone was originally discovered in 1928 by Bürger, was called "glucagon" by Murlin in 1929, and was designated the "hyperglycemic-glycogenolytic factor" by Sutherland and de Duve in 1948. Glucagon is antagonistic in its action to insulin. Thus, in experimental pancreatic diabetes [318 b, 366, 367] and in total pancreatectomy in humans [368–372], the requirement for insulin is considerably less than in alloxan diabetes in animals and in spontaneous human diabetes. The absence of pancreatic amylase and the diminished digestion of carbohydrates do not explain this fact [373]. Apparently, in human diabetes, part of the insulin required is needed to neutralize the anti-insulin hormone of the A-cells. The significant reduction of insulin requirement following pancreatectomy indicates that the pancreas contains such an anti-insulin substance, whose removal spares insulin. In certain birds (ducks), the amount of this substance may be so high that pancreatectomy is followed, not by diabetes, but by permanent, severe hypoglycemia which can be corrected by the administration of glucagon [374]. Glucagon (or HGF, "hyperglycemic-glycogenolytic factor") elevates the blood level of glucose at the expense of the glycogen of the liver. Glucagon is produced by the A-cells of the pancreas [277, 375] and perhaps also by the argentaffine cells of the stomach and duodenum (Gros-Schultze) [375–377]. The occurrence of glucagon in the latter cells is shown by the development of hypoglycemia following operations on the stomach [378] and by the beneficial effect of gastrectomy on diabetes [379].

The ratio of A-cells to B-cells in the pancreas is normally 1:4. In hyperinsulinism due to pancreatic islet adenoma, in spontaneous hypoglycemia, and in glycogen storage disease the ratio may fall to 1:20 or less [380–383]. In spontaneous human diabetes the ratio is 2:1 to 5:1 [348 b], and in alloxan diabetes it may be even higher [277]. It may thus be concluded that in both types of diabetes there is at least a relative, and sometimes an absolute, increase of glucagon production. This increased glucagon is also responsible, in addition to the decreased production of insulin, for the particular type of diabetic disturbance [384 a]. The relatively or absolutely increased production of glucagon is especially important in young, lean diabetics [385]. This concept is supported by the fact that the content of glucagon in the pancreas of diabetics is normal [386], while that of insulin is always diminished (p. 218). In addition, the injection of glucagon in oil into fed rats, rabbits, and dogs produces severe diabetes with marked glycosuria and loss of nitrogen [387]. As yet, it is not known whether the protein-catabolic action of glucagon is linked to a metabolic relationship between the adrenal cortex and the A-cells of the pancreas (see p. 228).

The role of glucagon in the etiology of diabetes was long in doubt because the injection of aqueous solutions of glucagon, while producing transient hyperglycemia,

would not produce a true diabetic state. This result is due to the fact that glucagon undergoes rapid enzymic degradation within the body, especially in the liver. The responsible enzyme is probably identical with insulinase, for the enzymic breakdown of glucagon in the liver is competitively inhibited by insulin [388]. An additional cause for the difficulty of producing experimental glucagon diabetes is the fact that the utilization of glucose in the peripheral tissues is not impaired by glucagon [389] and, in addition, the diabetogenic action of glucagon depends on the presence of rich reserves of glycogen in the liver [390]. If these reserves are used up to produce glucose, as by the repeated injection of glucagon in the rat, additional injections of glucagon fail to produce additional hyperglycemia but rather produce more and more hypoglycemia as the injections are continued. The resulting hypoglycemia may be severe enough to produce shock [391]. This paradoxical glucagon hypoglycemia is explained by the fact that glucagon, in addition to its action in mobilizing glycogen, also increases the utilization of glucose in the tissues [392, 393 a]. This latter action is prolonged and intense. There is no concomitant synthesis of glycogen in the extrahepatic tissues [393 b]. However, it is not yet clear to what degree the increased extrahepatic uptake of glucose is the result of a simultaneous, secondary outpouring of insulin. The dependence of glucagon hyperglycemia on the presence of glycogen in the liver is also shown by the fact that glucagon does not increase the blood sugar in hepatectomized animals [395] or in patients with liver disease [394, 410].

Conversely, when the A-cells of the pancreas are destroyed by specific cellular poisons such as synthalin or p-amino-benzol-sulfonamid-isopropyl-thiodiazol (IPTD), the blood sugar falls in proportion to the dose of the cellular poison, and may even fall to shock levels [396, 397]. This fact again indicates the importance of the A-cells and glucagon in maintaining the normal level of blood glucose. The hypoglycemia which follows damage to the A-cells is associated with an almost complete disappearance of the glycogen of the liver, indicating that the metabolic phenomena following A-cell damage are determined by a functional excess of insulin. If, in the rat or the rabbit, both the A-cells and the insulin-producing B-cells are destroyed (the latter by alloxan), this excess of insulin disappears, and the animal shows a carbohydrate pool which resembles that of the normal, but is abnormally labile. Thus, glucagon and insulin compensate for each other in their biological actions, and normal glucose balance is possible only when both hormones are present or both hormones are absent [396]. Alloxan diabetes, which is due to damage of the B-cells with resulting non-production of insulin, is prevented or improved if the A-cells are also damaged by synthalin or IPTD, as mentioned above [396, 397]. In contrast to these A-cell poisons, the arylsulfonyleurea derivatives have no effect on alloxan diabetes [398, 399], so that their site of action cannot, as has been suggested [400], be the A-cells. Histologic studies confirm this conclusion [398, 401].

This reciprocal relationship between insulin and glucagon need not be a simple unlimited antagonism between the two hormones. The observations that both substances increase the peripheral utilization of carbohydrates and reduce the serum levels of phosphate and amino acids [402] and reduce the liver glycogen make such an interpretation inadequate. In 1929, Bürger suggested that the relationship of the two substances was that of a mutually reciprocal effect insofar as glucagon affects glucose by glycogenolysis while under the influence of insulin glucose is utilized [403]. In the postabsorptive period, glucagon acts by causing an increased production of insulin and can thus produce severe hypoglycemia under certain conditions. Conversely, small amounts of insulin can cause an outpouring of glucagon with subsequent hyper-

glycemia in the dog and in individuals with a low fasting blood sugar [297, 393 a]. In the individual with a normal pancreas, on the other hand, the action of endogenous or injected insulin is reduced by such outpouring of glucagon, and conversely [336, 404].

Glucagon also has a reciprocal relationship to the hormones of the adrenal cortex. Glucagon increases the excretion of corticoids in the urine [405]. The injection of glucagon causes an initial fall of liver glycogen, which is followed by an increase of liver glycogen in normal rats, but not in adrenalectomized and hypophysectomized rats [406]. This action is mediated by a secondary activation of adrenal cortical hormones, which may explain the hyperactivity of the adrenal cortex in "counterregulation diabetes." The fact that insulin diminishes the action of glucagon establishes the differential diagnosis between juvenile insulin-deficiency diabetes and counterregulation diabetes by the use of a glucagon blood sugar curve. In insulin-deficiency diabetes, prior treatment with insulin results in a reduction of the glucose rise following the injection of glucagon. In patients with counterregulation diabetes, on the other hand, which occurs despite the presence of adequate stores of insulin, the administration of insulin has no effect on the hyperglycemia produced by the subsequent injection of glucagon [407].

The mechanism of action of glucagon is known only as regards its action on the liver. The glycogenolytic action of glucagon is due to the activation of the phosphorylases of the liver [408]. In glycogen storage disease, where the enzyme glucose-6-phosphatase is lacking in the liver (Cori and Cori), glucagon fails to cause a rise of blood sugar because glucose cannot be set free from its phosphorylated precursors and from glycogen [409, 410, 411]. This is called a "negative glucagon test." Glucagon both increases glycogenolysis and inhibits glycogen synthesis in the liver [412]. In addition, it inhibits the synthesis of fatty acids in the liver [413, 420]. A few studies also suggest that glucagon also inhibits the synthesis of cholesterol [414], but these have not yet been confirmed.

Epinephrine

The importance of epinephrine in the chemistry of diabetic metabolic processes is generally exaggerated. Epinephrine has been shown, however, to counteract the effect of insulin, and each injection of insulin causes prompt and rapid outpouring of epinephrine. This effect is responsible for the biphasic nature of the blood glucose curve which occurs after the prior injection of insulin [339, 341, 415, 416] (p. 224), but there is no evidence that diabetic hyperglycemia and glycosuria are at all due to an increased production or increased activity of epinephrine. This is unlikely in any case, since epinephrine is rapidly broken down within the body by the enzyme aminoxidase (Weil-Malherbe). The hyperglycemia and glycosuria which can be produced in acute experiments by the injection of epinephrine depend on the fact that epinephrine increases the amount of active phosphorylase in the liver and muscles by increasing the conversion of inert phosphorylase B to active phosphorylase A. The result is increased breakdown of glycogen, which has been shown in the intact animal [417], in isolated muscle [418, 419], and in liver slices [408]. The breakdown of glycogen is followed by an increase of glucose-6-phosphate and fructose-6-phosphate [421]. These two esters inhibit hexokinase [422] and, by blocking this enzyme, cause a diabetic state of metabolism (increased production of glucose in the liver, reduced utilization of glucose in the tissues) [423, 424]. On the other side, the reduced oxidation of glucose in the muscles which follows the administration of epinephrine

causes an increase of lactic acid, which passes into the blood and into the liver, where it is reconverted to glycogen, replacing the glycogen loss by glycogenolysis [425]. Epinephrine thus does not alter the liver glycogen significantly, despite the increased glycogenolysis, in contrast to the effect of glucagon (p. 226).

Of perhaps greater importance in the etiology of diabetes is the fact that epinephrine can have a diabetogenic effect on carbohydrate metabolism by stimulation of the anterior pituitary gland in stress situations.

Hormones of the Adrenal Cortex and ACTH

The first indication of the importance of the adrenal cortex in the genesis of diabetes was discovered over 20 years ago. At this time, it was found that the injection of extracts of adrenal cortex aggravated the metabolic disturbance of pancreatectomized dogs [426] and that adrenalectomy could improve pancreatic and alloxan diabetes in the cat and the dog [427]. Even before this time, clinicians had been impressed with the frequency of the coincidence of diabetes and adrenal cortical adenomas [428] and with the finding that insufficiency or ablation of the adrenals improved spontaneous human diabetes [429, 430]. In the partially pancreatectomized rat, administration of extracts of adrenal cortex or of cortical hormones exacerbates diabetes previously improved by adrenalectomy [431, 432] and aggravates diabetes in animals with intact adrenal glands [431, 433]. Another similar observation is the finding that adrenalectomized alloxan diabetic animals show increased sensitivity to insulin or reduced requirement for insulin [434]. Conversely, the injection of diabetogenic adrenal cortical hormones causes resistance to insulin, and this must be distinguished from the insulin-resistance produced by the growth hormone of the anterior pituitary or by anti-insulin antibodies [436 a].

The diabetogenic activity of cortical extracts is due to corticosteroid hormones, especially cortisone and hydrocortisone, while corticosterone and 11-dehydrocorticosterone are much weaker in action [431] (Table 54). Progesterone also has a weak diabetogenic action, which is explained by the conversion of progesterone into corticosteroids in the adrenal cortex [436 b]. Stronger actions are exhibited by 11-hydroxyprogesterone and 11-ketoprogesterone (Table 54). The synthetic hormones prednisone, prednisolone, and the fluoridated corticosteroids also show marked diabetogenic activity. All these hormones are grouped together as "glucosteroids." Carbohydrate metabolism is similarly influenced by corticotrophin (ACTH), whose diabetogenic effect is mediated by the adrenal cortex, so that it may be discussed together with the hormones of the cortex. The diabetogenic activity of the glucosteroids and of ACTH is especially pronounced in the rat, guinea pig, rabbit, and the Rhesus monkey, and results in glycosuria, hyperglycemia, increased production of glycogen in the liver, and negative nitrogen balance [433, 435, 437-441, 512]. It is less clear in the dog and the cat, which show marked resistance to the diabetogenic action of the glucosteroids. The dog is also protected by cortisone from the diabetogenic effect of somatotrophic hormone (STH) [442]. The reason for this species specificity of cortisone is not known.

Man reacts to cortisone in the same way as the rat, showing, according to the amount of cortisone given, a prediabetic stage, recognizable only by the glucose tolerance test; or overt diabetes, which develops either by itself (steroid diabetes) or in combination with insulin deficiency diabetes [436 a, 443]. In this second instance, mild diabetes can be made worse or latent insulin deficiency can become manifest [444, 445]. The development of overt diabetes can follow every type of stress which

increases the production of adrenal cortical hormones (e. g., inflammation, trauma) [446 a, 446 b]. The elevation of blood sugar which is necessary for the normalization of glucose-utilization in insulin-deficiency diabetes can be attained only by increased formation of glucose; i. e., by stimulation of the adrenal cortex. Each episode of hyperglycemia in itself causes an outpouring of glucosteroids from the adrenal cortex [354]. This fact supports the concept that in severe or progressive diabetes, hyperfunction of the adrenal cortex is superimposed on what originally was pure insulin-deficiency. In alloxan diabetes, such a combination of insulin deficiency and cortical hyperactivity has already been established by histologic and biochemical studies [122, 124, 125, 447, 448, 449, 450]. Chemical disturbances of the zona fasciculata, where the glucosteroids are produced, improve the hyperglycemia caused by alloxan despite concomitant degeneration of the B-cells of the pancreas [451], demonstrating the dependence of alloxan hyperglycemia on the adrenal cortex. The pancreatic diabetes of the dog also shows increased adrenal cortical hyperactivity [452].

Even in phlorhidzin diabetes in the rat, which is ameliorated by adrenalectomy and aggravated by cortisone, there is superimposition of a glucosteroid effect [453]. In human diabetes, extreme hyperfunction of the adrenal cortex is presumed to be present in the stage of acidosis and precoma even in those cases in which pure (juvenile) insulin-deficiency diabetes was previously present. This is even truer in older patients

Table 54. Diabetogenic Glucosteroids

No.	Name of Glucosteroid	Test Used					
		Synthesis of liver glycogen Rats			Glucose tolerance Rats	Production of glycosuria	
		(a)	(b)	(c)		Rats	Man
		(a)	(b)	(c)	(d)	(e)	(f)
1	9 α -Fluoro-2-methylhydrocortisone	38					
2	9 α -Fluoroprednisolone			50	20		20
3	2-Methylhydrocortisone	10					
4	9 α -Fluorohydrocortisone		10.7	13	15		20
5	9 α -Fluorocortisone		9				
6	9 α -Fluorodehydrocorticosterone		5.9				
7	9 α -Chlorohydrocortisone		4.7				
8	9 α -Fluorocorticosterone		4.6				
9	9 α -Chlorocortisone		3.7				
10	Prednisolone				4		4
11	Prednisone				3		
12	11 β ,17-dihydroxyprogesterone					ca. 2	
13	9 α -Fluoro-11,17-dihydroxyprogesterone		1.9				
14	Hydrocortisone	1		1	1	1	1
15	Cortisone		1		0.7		
16	11 β -hydroxyprogesterone		0.2			ca. 1	
17	11-Ketoprogesterone					ca. 1	
18	9 α -Fluoro-11 β -hydroxyprogesterone		0.85				
19	Corticosterone				0.3		
20	Aldosterone				0.2		
21	11-Dehydrocorticosterone				0.1		
22	Progesterone		+				+

The numbers give the relative activities compared to a value of 1 for cortisone or hydrocortisone, respectively. The letters (a) to (f) refer to bibliography references 665 a to 665 f respectively.

with sthenic builds and relatively good insulin reserves – “maturity-onset diabetes” where the metabolic processes are characterized by the production of diabetogenic glucosteroids [454–456]. In childhood diabetes, which is an especially severe form of insulin-deficiency diabetes, adrenal cortical hyperfunction is always present [497], even when insulin controls the manifestations of diabetes. In diabetic children aged 4 to 16 years, the serum corticoid level has been found to be the more elevated, the more marked the glycosuria, acetonuria, and the reduction of the alkali reserve.

This cortical hyperfunction in prolonged diabetes may lead to the development of severe late complications (Kimmelstiel-Wilson syndrome, retinopathy) [457, 458, 459 a]. The causal relationship between the increased activity of the adrenal cortex and the development of renal glomerulosclerosis is supported by the observation that prolonged administration of cortisone or ACTH produces changes resembling those of Kimmelstiel-Wilson syndrome even in non-diabetics [459 b]. Prolonged administration of cortisone, hydrocortisone, chlorohydrocortisone, or ACTH causes intercapillary glomerulosclerosis in normal rabbits and in rabbits with alloxan diabetes, the lesions resembling those seen in the human Kimmelstiel-Wilson syndrome [460–462]. Conversely, adrenalectomy [464] or hypophysectomy [465] can reverse the symptom complex of glomerulosclerosis and retinopathy, if the changes are still reversible. In cases of diabetic nephropathy, the adrenal glands show an increased weight and an increased incidence of cortical adenomas. Conversely, chemical depression of glucosteroid production by DDD (2,2'-bis-(p-chlorophenyl)-1,1-dichlorethane) improves the hyaline changes of the blood vessels of Kimmelstiel-Wilson syndrome [463]. Recent observations suggest that there is not only an increased amount of corticosteroids in diabetes, but that there are qualitative changes of these hormones, for cortisone in diabetics is converted to 17-ketosteroids, in contrast to the normal [466]. It may be that it is not cortisone itself but an abnormal degradation product of cortisone which is responsible for glomerulosclerosis.

The diabetogenic action of an increased amount of adrenal corticosteroids in man, however, may be seen in another type of diabetes, “steroid diabetes.” In “counter-regulation diabetes” (“maturity onset diabetes”) the increase of glucosteroids is secondary to an underlying insulin-deficiency state. In steroid diabetes, on the other hand, there is pathological hyperactivity of the adrenal cortex which is primary in nature and is in no way related to the pancreatic islets. Cushing’s disease, which has been known for a long time, always shows at least a diabetic type of glucose tolerance test, and in many cases frank hyperglycemia and glycosuria are also present [445]. Under certain circumstances, such as adenomas of the zona fasciculata, the amount of glucosteroids produced can be so great that severe diabetes with marked glycosuria and marked loss of nitrogen develops. In these cases, the urine contains large amounts of hydrocortisone [467]. In the same way, prolonged administration of cortisone or ACTH may produce steroid diabetes [446 b, 468]. This form of diabetes shows marked resistance to insulin, severe glycosuria in the presence of only mild hyperglycemia, increased amounts of lactate and pyruvate in the blood, a tendency to improvement during starvation, a tendency to the deposition of fat, and secondary hypertrophy of the islands of Langerhans with increased production of insulin [469, 470, 471, 472]. This compensatory overproduction of insulin explains the tendency of steroid diabetes to spontaneous remission [472].

Diabetes in Pregnancy. An important form of steroid diabetes occurs during pregnancy and in children of diabetic mothers. During pregnancy, the body contains increased amounts of cortisone and hydrocortisone, so that the blood level of these

hormones reaches 13-23 mg% (normal < 1 mg%) [474] and increased amounts are excreted in the urine [475]. These hormones are formed in increased amounts in the hyperactive adrenal cortex and are also produced by the placenta [473]. They cause an increase of the glycogen content of the placenta and thus supply glucose to the fetus. Sometimes, however, they may be produced in such excessive amounts that frank diabetes may result. Secondary exhaustion of the islands of Langerhans may then produce a permanent type of diabetes ("metagestational diabetes") [476], although the diabetogenic action of prolactin may be partly responsible for this permanent diabetes (p. 240). The amniotic fluid of pregnant diabetic women contains a greater quantity of corticosteroids than normal [477]. Children born of diabetic mothers excrete increased amounts of 11-hydroxy- and 11-deoxycorticoids in the urine during the first week of life, as well as increased amounts of 11-ketosteroids formed in the adrenal cortex [478]. They are also abnormally sensitive to the action of ACTH [478]. Hyperfunction of the adrenal cortex in such children is also demonstrated by their increased weight, their Cushing-like facies, and a tendency to edema and hypokalemia [478]. However, prolonged diabetes does not occur in these children because there is compensatory hyperplasia of the B-cells of the pancreas [479].

"Metacorticoid Diabetes." If hyperfunction of the adrenal cortex continues for a long period of time or if cortisone is administered over a long time, the initial steroid diabetes may cause overloading and damage of the B-cells of the pancreas and lead to a permanent "metacorticoid" diabetes which persists despite cessation of steroid activity [326, 480]. In practice, it is difficult to distinguish between these two types of diabetes.

Pathogenesis of Steroid Diabetes. The diabetogenic action of the glucosteroids and of ACTH is due to increased gluconeogenesis from non-carbohydrate substances [431, 481] (p. 202). The mechanism of increased gluconeogenesis by the corticosteroids has been studied by isotopic technics (Table 50). The first substance formed is glucose-6-phosphate (p. 204): cortisone increases the amount of glucose phosphates in the liver and muscles [482]. Glucose-6-phosphate can then be metabolized in two different ways. *Some is converted in increased amounts into free glucose*; the enzyme which catalyzes this reaction – glucose-6-phosphatase – shows increased activity in the liver under the influence of the glucosteroids, especially cortisone and hydrocortisone [190, 251, 483]. The same is true in alloxan diabetes, where there is also an increased production of the corticosteroids [251]. The hyperglycemia of steroid diabetes is explained in this way.

The rest of the glucose-6-phosphate is converted to glycogen and stored [431, 484], so that the extent to which the corticosteroids are responsible for the development of diabetes can be estimated from the amount of glycogen deposited in the liver [431, 485-487]. This reaction explains the increased liver glycogen in alloxan diabetes [122, 124, 125, 447-449] and in experimental steroid diabetes [488]. The liver glycogen is greater the greater the dose of cortisone and the more severe the glycosuria. The disappearance of liver glycogen following adrenalectomy in alloxan diabetes is also explained by these observations [125]. The increased amounts of glycogen in the liver of older diabetics has been repeatedly noted [489]. The increased liver glycogen following cortisone is due to several mechanisms: (1) increased gluconeogenesis; (2) inhibition of glycogenolysis [487, 490], perhaps controlled by glucomutase [491]; and (3) inhibition of carbohydrate oxidation in the liver [492]. The glycogen of muscle, in contrast to that of the liver, is little affected by the glucosteroids [431]. The *in vitro* inhibition of glycogen synthesis by steroids found in extrahepatic tissues is

probably a nonspecific surface effect [493, 494]. An inhibitor effect of cortisone on muscle phosphorylase has also been used to explain the influence of the hormone on muscle glycogen, but this effect is also a nonspecific one, for it is also shown by progesterone and testosterone [495].

The basic biological effect of the glucosteroids of the adrenal cortex, *gluconeogenesis*, occurs by three mechanisms. (1) One source of the glucose and glycogen formed by gluconeogenesis consists of *intermediary products formed in glycolysis*, notably lactic acid and pyruvic acid. Adrenalectomy diminishes the synthesis of liver glycogen from lactate and pyruvate [420, 435, 496-498]. This process of gluconeogenesis from pyruvate, which in essence is a hormonally governed reversal of glycolysis, occurs by virtue of the fact that an excess of pyruvate is formed from the citric acid cycle and from amino acids, so that more pyruvate passes through the synthesizing chain of glycolytic enzymes. Elevation of the blood level of pyruvate occurs regularly in steroid diabetes [463, 499] because of the energy requirement of the pyruvyl kinase reaction (this can be reversed only by the shuttle pathway), so that pyruvate tends to accumulate.

(2) The second and physiologically most important source of newly formed carbohydrate is *protein* [431, 433, 438]. In the first stage of the process of gluconeogenesis from protein, increased amounts of body protein are degraded and converted to intermediary products of carbohydrate metabolism ("protein catabolic effect"). The nitrogen thus set free is excreted as urea or uric acid (from nucleoprotein) [500]. The glucosteroids and ACTH thus cause an increase of the amino nitrogen in the blood and an increased excretion of nitrogen in the urine, with resulting negative nitrogen balance [431, 438, 500, 501]. They also result in diminished deposition of protein, which in turn may result in impaired growth [502, 503]. These effects are seen in mammals in general, as well as in man, and they occur to the degree that the body protein undergoes the above conversions. Conversely, adrenalectomy reduces the blood level of amino acids and increases the deposition of protein (i. e., promotes growth) [503-506]. The key process in the conversion of protein to carbohydrate under the influence of the corticosteroids is deamination or transamination of amino acids to nitrogen-free products (α -ketoacids), which can serve as intermediary products in carbohydrate synthesis. The preceding proteolysis is merely preliminary, and its intensification by the corticosteroids is merely secondary in nature [507, 508].

The synthesis of carbohydrates from "glucoplastic" amino acids (p. 205) is blocked both in vitro and in vivo by adrenalectomy [435, 509, 510]. Conversely, cortisone increases the glycogen of the liver to a degree which is proportional to the fall of the liver content of aspartic acid and glutamic acid [511], the glutamic acid undergoing oxidative deamination or transamination under the influence of the corticosteroids [513]. The reduction of blood glutathione in steroid diabetes is related to these metabolic events [500, 514, 515]. It may therefore be postulated that the corticosteroids somehow activate the deaminating and transaminating enzymes of the liver and the kidneys [129]. Since some of these enzymes require pyridoxal phosphate for their action, it can be concluded that increased amounts of pyridoxal phosphate are needed in the presence of adrenal cortical hyperfunction. Hence the danger of pyridoxin deficiency under such conditions, including diabetes. The fact that pyridoxin reduces the glycosuria and loss of nitrogen in alloxan diabetes [516] is in agreement with this concept. The existence of pyridoxin deficiency in diabetes is confirmed by the changes in tryptophane metabolism, which are typical of those seen in pyridoxin deficiency. Thus, in the normal individual, ingested tryptophane is converted into nicotinic acid and N-methylnicotinamide under the action of pyridoxin. When pyridoxin is

lacking, the degradation of 3-hydroxy-anthranilic acid is blocked, and tryptophane is converted to pathological substances including kynurenic acid and xanthurenic acid. This situation is actually seen in human diabetes and in alloxan diabetes in animals. In alloxan diabetes, the administration of a loading dose of tryptophane is followed by the appearance in the urine of markedly subnormal amounts of N-methylnicotinamide [517], and instead the urine shows 3-hydroxy-kynurenin [518], picolinic acid [519], and xanthurenic acid [518, 520] – i. e., substances found in pyridoxin deficiency. In human diabetes, ingestion of a test dose of 10 grams of DL-tryptophane is followed by the appearance of xanthurenic acid in the urine in abnormally large amounts (33 mg/day in contrast to the normal 10 mg/day) [520]. The situation can be returned to normal by the administration of pyridoxin. Insulin, by decreasing gluconeogenesis, has the same effect as pyridoxin [519, 521, 526]. Adrenalectomy, by eliminating the pyridoxin-utilizing conversion of protein to glucose, reduces the excretion of kynurenic acid in the urine; cortisone increases this excretion [522]. The depletion of the body content of pyridoxin because of the intense gluconeogenesis is also shown by the excretion of cystine and methionine in the urine of diabetics [523 a], for these two amino acids can be utilized by the body only in the presence of adequate amounts of pyridoxin. All these signs of pyridoxin deficiency in diabetes are thus the result of the increased synthesis of glucose from proteins and amino acids, a synthesis which is due to the corticosteroids and which demands the presence of pyridoxin.

(3) The third source of newly-formed glucose is *fat*, whose conversion to glucose is also under the influence of the adrenal cortical hormones [523 b]. The relationships of these hormones to fat metabolism are complex and poorly understood. In the normal starved animal cortisone reduces the amount of ketone bodies in the blood [524, 525], thus exerting an antiketogenic effect. If, however, the breakdown of carbohydrates and the formation of antiketogenic metabolites are already disturbed, as is the case in pancreatic and phlorhidzin diabetes, cortisone and ACTH increase the production of the ketone bodies [433, 527, 530], while adrenalectomy has the opposite effect [529]. In man, cortisone always increases the production of ketone bodies [436 a, 445, 528]. This ketogenic effect in man and in animals is part of the process which converts fat into glucose by way of active acetate. At the same time, cortisone and ACTH inhibit the conversion of carbohydrates into fat and thus indirectly promote gluconeogenesis [527], thus sparing newly formed carbohydrate. On the other hand, 11-dehydro-corticosterone and perhaps other pathological steroids formed in diabetes seem to have a lipogenic function [531, 532]. The fatty liver which follows the use of cortisone is merely the result of an increased transport of fat to the liver, the site of gluconeogenesis.

Cortisone increases the synthesis of *cholesterol* from acetate [533, 534] and undoubtedly plays an important role in the etiology of the disturbances of cholesterol metabolism which are present in diabetes.

Decreased Utilization of Glucose. In addition to the increased gluconeogenesis, there is also diminished utilization of glucose in the peripheral tissues in diabetes. This action is part of the diabetogenic effect of the corticosteroids, and is presumably due to an anti-insulin action. In the normal individual, this reduced utilization of glucose can be compensated for by increased activity of the B-cells of the pancreas. This compensation does not occur in the patient with insulin-deficiency diabetes. The peripheral anti-insulin effect consists of inhibition of glucose uptake by the cells [226, 535] and of phosphate uptake by the cells [536], and of inhibition of the formation of muscle glycogen [537]. The effect is not due to any direct action of the corticosteroids,

for these do not inhibit the oxidation of glucose to CO_2 in diabetogenic doses [481, 538]. The anti-insulin effect rather seems to be due to a protein which has the characteristics of a β -lipoprotein and which is synthesized in the liver under the influence of cortisone in the presence of the growth-hormone of the anterior pituitary [539, 624]. This lipoprotein specifically inhibits the transfer of glucose across the cell membrane [540], and is thus a typical anti-insulin (p. 239). It is not, however, completely neutralized by insulin, suggesting that it has an additional site of action which is not related to insulin [541]. This additional effect seems to consist of a true inhibition of hexokinase [542] and is difficult to distinguish from the anti-insulin effect. In 1945, Corn and coworkers therefore suggested that the action of insulin is against hexokinase (p. 212). The hexokinase-inhibiting β -lipoprotein (Bornstein's "secondary humoral factor") is found in the blood in human and alloxan diabetes [539, 542, 543, 624]. It must be differentiated from anti-insulin antibodies, which are found among the γ -globulins [544, 545]. The synthesis of the β -lipoprotein in the liver may explain the improvement of the glucose tolerance curves and amelioration of diabetes in diabetics with liver disease.

It is characteristic of the steroid forms of diabetes that the degree of glycosuria is much greater than the usually slight hyperglycemia would suggest and that glycosuria may appear when the glucose tolerance and the blood sugar are still normal [546]. This fact suggests a renal site of action of the corticosteroids. It has been shown that cortisone [547, 548] and ACTH [548-550] reduce the renal threshold of glucose by 50%, because of an increase of glomerular filtration with relative or absolute reduction of tubular reabsorption [548]. The renal glycosurias of pregnancy, which may pass into true diabetes, are largely mediated by the adrenal glands. In analogy to the situation with regard to insulin and glucagon, the corticosteroids also call forth counter-corticosteroid mechanisms which reduce their diabetogenic action and help return carbohydrate metabolism towards a homeostatic mean. The most obvious expression of this counter-regulation is the activation by cortisone of the pancreatic islands with marked morphologic increase of the B-cells [479, 551, 553 a], but without any change of the A-B relationship. The increased production of insulin can result in paradoxical sparing of insulin following the treatment of diabetics with cortisone [639 a], but the continued action of cortisone leads to exhaustion of the B-cells and to so-called "metacorticoid diabetes" (p. 223).

STH (Somatotrophic hormone, Somatotrophin, Growth hormone of the anterior pituitary)

In 1887, Pierre Marie first commented on the frequent occurrence of diabetes and acromegaly in the same individual. Marie and later workers (Atkinson, Coggeshall and Root) noted that diabetes occurs in one-third of patients with acromegaly. The suggestion that STH had diabetogenic properties was in agreement with the observation that pancreatic diabetes could be ameliorated by hypophysectomy, the so-called "Houssay effect" [553 b], and was confirmed by the experimental demonstration in 1945 that pure STH produces diabetes in the dog [322, 323, 554, 555]. At the same time, it was shown that the anterior pituitary intensified diabetes in at least two ways, by means of corticotrophin, which acts only via the adrenal cortex, and by STH, which works even after adrenalectomy. While ACTH is especially diabetogenic in the rat, rabbit, and guinea pig, the diabetogenic effect of beef STH is seen chiefly in the adult dog and cat and is absent in the Rhesus monkey. In rodents, the diabetogenic effect

of STH occurs only if the function of the B-cells has previously been reduced, for example by partial pancreatectomy or following alloxan in subdiabetogenic doses [556, 557, 563].

In man the situation is analogous: ACTH produces diabetes by stimulating the adrenal cortex (p. 235), while commercial STH produces a detectable diabetogenic effect only when prior injury to the islands of Langerhans has produced a prediabetic state, or when corticotrophic activity is already increased. In human acromegaly, diabetes develops only if the secretion of ACTH has not been eliminated [558], and the diabetogenic action of STH in man occurs only on the basis of a prediabetic state (increased secretion of ACTH) [559]. In the rat also ACTH or the glucosteroids which it stimulates potentiate the diabetogenic action of STH [560, 561]. In the dog and the cat, on the other hand, the physiological secretion of ACTH suppresses the diabetogenic action of parenteral STH, and conversely [562]. The antagonism between STH and ACTH in the dog and the cat is thus in contrast to the mutual increase of action shown by these two hormones in the rat and in man. The type of correlation between the two hormones seems to depend on the responsiveness of the pancreatic islands and especially on the reaction of the B-cells to excessive stress [561]. Thus, the resistance of the rodent to STH probably depends on its permanent production of insulin, which cannot be exhausted even by tremendous secretory stimulation. In the dog and the cat, on the other hand, the secretion of the B-cells is rapidly exhausted on prolonged stimulation [565, 566].

The different responses of different species of animals as regards the action of STH are primarily due to the fact that STH is not one single substance, but is a protein whose structure depends on the species and is species-specific [564]. The action of this STH protein can thus be correctly studied only in homologous species. In Rhesus monkeys, for example, an effect on carbohydrate metabolism occurs only when Rhesus STH is administered, but not if the source of STH is beef or hog [556]. The same is true in man, who responds with diabetes when his own STH is increased in acromegaly or when homologous STH is administered [678], but fails to respond to commercial beef STH. These facts are too often overlooked in discussions of the diabetogenic action of STH, increasing the difficulties of explaining this action. In addition, there are many other effects of this hormone, not all of which are known. It is essential to differentiate between true "idiophyphyseal" STH diabetes, which occurs after short term administration of STH, and the permanent or "metahyphyseal" diabetes which occurs after prolonged treatment with STH [323, 555, 567]. The latter is a true insulin-deficiency diabetes (p. 223). At this point, we shall discuss only the first type, idiophyphyseal diabetes.

To understand this type of diabetes, which is caused by overproduction or overdosage of STH, it is necessary to have detailed and precise analyses of the metabolic effects of this hormone. Much of the controversial literature on STH lacks such analyses and therefore records not only direct actions of STH itself but also actions which are mediated indirectly by other hormones.

Actions of STH. The most important, perhaps the only, direct metabolic effect seen in human beings after the injection of pure STH is *increased breakdown of fats to ketone bodies* [568]. This action seems to depend on the fact that STH provides the coenzyme A which is necessary for the β -oxidation of fatty acids, apparently by promoting the synthesis of coenzyme A [569]. The increased production of ketone bodies in severe diabetes is the result of increased production of STH. STH increases the needs for pantothenic acid, which is necessary for the synthesis of coenzyme A [570], and

increases the liver content of coenzyme A [569]. STH is inactive when pantothenic acid is deficient [571]. The natural sign of this coenzyme A effect is an increase of the fat of the liver. This is the expression of increased transport of fat from fat depots to their site of breakdown, with an increase of the ketone bodies in the blood in man, the rat, and the mouse [572, 573, 575]. The well-known deposition of fat which occurs in animals following hypophysectomy [574] can be considered the reverse of this effect. The livers of rats treated with STH show an increased activity of fatty acid oxidases and therefore produce two to three times as much acetoacetic acid as normal, untreated rats [575, 576]. The acetoacetic acid is apparently formed by recondensation of acetate moieties, which cannot enter into the normal pattern of catabolism in the citric acid cycle because of the concomitant cessation of carbohydrate oxidation.

The increased breakdown of fatty acids under the influence of STH is independent of the adrenal cortex [577]. It is accompanied by a corresponding fall of the respiratory quotient and leads – as a result of an increase of H atoms liberated by the dehydrogenation reactions of fat catabolism – to an increased synthesis of reduced coenzymes (DPNH) [578]. This “high fat metabolic pattern” which produces increased fat deposition as the initial effect of STH also causes *deposition of carbohydrate and protein*. Thus, less carbohydrate is oxidized than normally [579], without any qualitative change in the individual reactions of carbohydrate metabolism [537], and more carbohydrate is stored as glycogen in the skeletal muscles [580] and the muscle of the heart [581]. This action of STH is not dependent on the function of the adrenal cortex [582]. In starved animals, this “myoglucostatic” action of STH produces no change in the glycogen stores, but in fed animals it increases these stores. The reaction is not influenced by insulin [537, 583]. STH and insulin are synergistic as regards their tendency to maintain tissue glycogen, but not with regard to their effect on the oxidation of carbohydrates. Cortisone in subliminal doses is necessary for the complete myoglucostatic effect of STH: this is the “permissive action” of cortisone described by Ingley [584]. The myoglucostatic action of STH, which blocks the oxidative breakdown of glucose at the expense of oxidation of fat, contributes to the total diabetogenic action of STH discussed below.

An effect similar to that exerted on carbohydrate metabolism is also seen in the case of protein metabolism: The “high fat metabolic pattern” of the direct action of STH, with its preference for fatty acid breakdown, pushes protein metabolism in the direction of storage [568]. STH reduces the breakdown of proteins, inhibits proteolysis and the production of urea and ammonia [585, 586], and causes a fall of the residual nitrogen and marked diminution of nitrogen excretion in the urine in the rat and in man [588, 589]. It also causes a reversal of the direction of protein metabolism, so that proteins are synthesized and stored in increased amounts [587, 590]. The energy required for the latter reactions is produced by the increased breakdown of fats. The direct dependence of the increased protein synthesis on the increased breakdown of fats is shown by the fact that, when the fat depots are depleted, STH no longer promotes growth [572]. There is also a considerable increase of the synthesis of proteins from amino acids, the “protein anabolic effect” of STH, which results in increased muscle protein [596] and true growth [589, 598]. This effect [585, 591] is only partially independent of insulin and of the presence of B cells [592, 593, 595], and takes place largely in the liver [594]. The concentration of total plasma proteins per unit body weight shows a significant rise under the action of STH, and the weights of the liver and kidneys increase [565].

These growth promoting and protein depositing actions of STH are of interest in diabetes, although they are incompletely understood. In all likelihood, the excess non-

utilizable protein formed under the influence of STH is converted into glucose [597]. Usually, a sudden increase in growth precedes the onset of juvenile diabetes, only to stop when the diabetes becomes manifest [599]. The large size of infants born of diabetic mothers is apparently due to overproduction of STH. In pregnant rats and dogs, the administration of STH also produces excessively large offspring (fetal gigantism) [600, 601]. The level of STH in the blood plasma of pregnant rats is three times as high as that of non-pregnant rats, and is reduced toward normal by hypophysectomy, suggesting that some of the STH is produced in the placenta or the fetal hypophysis [602].

All these primary effects of STH do not cause diabetes directly, but lead to an increase of carbohydrates and proteins, substances whose turnover during insulin-deficiency or in the presence of glucagon can lead to a diabetic type of metabolism. These latter conditions can result from the many effects which STH exerts on the endocrine system. The actual diabetogenic action of STH is thus the result of these indirect effects of this hormone.

Some of the metabolic effects of STH are mediated by the islands of the pancreas. This is especially true for the growth-promoting action of STH, which depends on the presence of adequate amounts of endogenous or exogenous insulin. Radioactive STH labelled with I^{131} is fixed by the pancreas after injection [603]. Even before the "high fat metabolic pattern" discussed above, STH causes an acute outpouring of insulin [604]. Under the action of STH, the pancreatic content of extractable insulin in the dog falls to trace levels (8.1 to 0.52 units/kg) [605]. At the same time, the insulin activity of the blood is increased, but not after pancreatectomy [606, 607 a]. These findings have been observed in the dog, the cat, and man. In acromegaly, the plasma insulin is significantly elevated; in panhypopituitarism, it is reduced [608]. In the rat, corresponding effects of STH have not been unequivocally shown [607 a]. The outpouring of insulin is manifested by typical insulin effects: increased uptake of glucose in the muscles [609, 622], increased oxidation of glucose in the muscles [609, 622], improvement of the glucose tolerance test [610], transient cessation of oxidation of fat [572], intensification of protein deposition and growth [207 a], and a fall of blood sugar which is dependent on insulin, for it vanishes after depletion of the B-cells of the pancreas. In the starved rat, the injection of STH is rapidly followed by hypoglycemia, which may reach dangerous levels after elimination of the gluconeogenetic function of the adrenal cortex by adrenalectomy [584]. The hypoglycemic effect is absent after pancreatectomy just as is the nitrogen retention caused by STH [590, 604], so that it is really due to increased production of insulin by the B-cells [611]. After STH, the islets of the rat show hypertrophy and degranulation, indications of increased activity of the B-cells [607 b, 612, 613].

However, the hypoglycemia following STH is due not only to an increase of insulin within the pancreas, but also to increased liberation of tissue insulin from its bound form in the tissues [614]. Thus, even in pancreatectomized animals there is still a tendency to acute hypoglycemia following the administration of STH [615, 616]. If the animals are first pancreatectomized and then left for several days without insulin, this tendency to hypoglycemia following STH can no longer be demonstrated [617]. This outpouring of insulin from the pancreas and the extra-pancreatic tissue depots is not due to a direct hormonal effect on the insulin-secreting cells, but rather to the fact that *STH increases the overall requirement for insulin by the peripheral tissues* [604, 618, 619 a]. This increased insulin requirement explains the beneficial effect of STH in patients with hyperinsulinism due to islet

adrenoma [619 b]. The increased tissue requirement for insulin is especially striking when there is no available insulin, as in pancreatic and metahypophyseal diabetes. In these cases, even small amounts of STH cause an acute exacerbation of the diabetes and increase the insulin requirement from four to ten times. The resulting insulin hunger apparently serves the purpose of concentrating more insulin in the tissues and thus, because of the protein-depositing potential of insulin [207 a], of promoting cell division and growth. When the production of insulin is diminished, there may be a disproportion between the insulin available and that necessary, the result being diabetes, chiefly as a result of the myoglucostatic function of STH, without impairment of peripheral utilization of carbohydrate. The tendency to the development of diabetes under the influence of STH can remain latent as long as the B-cells of the pancreas produce enough insulin to satisfy the increased insulin requirements of the tissues. In chronic administration of STH, and in overdosage with STH, this is not the case. The deficient saturation of the tissues with insulin produces such demands on the pancreas that the stage of overproduction of insulin with hypoglycemia is soon replaced by a stage of exhaustion of the B-cells, with hyperglycemia, insulin resistance, ketonemia, and glycosuria - i. e., true diabetes [316, 318, 620].

This reversible syndrome of so-called idiohypophyseal diabetes, which is characterized by degranulation of the B-cells and fall of the amount of insulin in the pancreas [565], can be produced by a few injections of large amounts of purified STH [322 b, 323]. It also develops when hypophysectomized-pancreatectomized (Houssay) animals are given STH [555]. This idiohypophyseal STH-diabetes is caused by the combined action of insulin deficiency and the specific "high fat metabolic pattern" effect of STH, and regresses spontaneously when STH is discontinued. In contrast to this is "metahypophyseal" diabetes, which is due solely to deficiency of insulin and is produced by prolonged administration of STH; this type of diabetes remains after STH is discontinued [322 a, 323]. Metahypophyseal diabetes shows atrophy of the islets of Langerhans and no production of insulin in these islets (p. 223). At the same time, the insulin activity of the plasma disappears, because of the formation of an insulin-binding protein in the plasma [621, 624]. Denaturation of this protein by repeated freezing and thawing of the plasma returns insulin activity to the plasma [606]. The production of a protein-like insulin inhibitor by STH explains the slow development of the anti-insulin action of STH [622] as well as the resistance to insulin which is characteristic of these idiohormonal types of diabetes. The hypophysectomized rat thus shows an extreme sensitivity to insulin which responds to the administration of STH [623]. The inhibitory substance can also be demonstrated in alloxan diabetic hypophysectomized cortisone treated rats after the injection of STH [624], and has been identified as a lipoprotein which belongs to the β_1 -globulins [539].

In the development of idiohypophyseal STH-diabetes, another secondary action, *that which influences the secretion of glucagon*, seems to be involved. Many observations show that purified STH increases the production of glucagon in the A-cells of the islets and increases the size, rate of mitosis, and secretory activity of these cells [552, 625 to 628]. The hyperglycemia and liver glycogenolysis which follow the intravenous injection of STH are probably due to the secretion of glucagon [629]. After the injection of STH, glucagon is present in increased amounts in the pancreatic veins of rats and cats [627], and the ratio of A-cells to B-cells is increased because of a relative and absolute increase of the number of A-cells [630]. The involution of the A-cells caused by hypophysectomy [631] is corrected by STH [632]. Isotopic studies suggest that STH is stored in the islet cells of normal and alloxan diabetic animals, probably in the

A-cells [633 a]. Conversely, damage or destruction of the A-cells is followed by the loss of the diabetogenic effect of STH [391, 633 b]. STH also stimulates the production of glucagon ("alphacytotropic factor" of Ferner), and thus acts to increase the total secretion of the pancreas. At first, the increases in activity of the A- and B-cells go hand in hand, but after prolonged overproduction of STH the B-cells may be overloaded and result in an isolated depletion of insulin formation. Together with the persistent secretion of the hyperglycemic glucagon, this may evoke frank diabetes.

Pluriglandular Correlations of the Anterior Pituitary

The diabetogenic effect of STH is not completely independent of other anterior pituitary hormones. An example of such dependence is the "permissive action" of subthreshold doses of ACTH on STH, so that, in hypophysectomized animals, the secretion of insulin due to STH is less than that in normal animals [650]. In addition, in such animals, STH elevates the level of insulin in the plasma only in the presence of another anterior pituitary hormone, which is not corticotrophin [606]. The production by STH of the lipoprotein which activates insulin is also dependent on the presence of small amounts of ACTH or cortisone [624]. Thus, the various hormones of the anterior pituitary mutually activate each other with regard to their action on carbohydrate metabolism. This is especially true for the pair STH-ACTH [561], in which the ACTH brings about the various gluconeogenetic effects via the adrenal cortex. *Prolactin*, which is also diabetogenic [330], also seems to work together with STH, causing the formation of milk during lactation [634]. The action of prolactin in carbohydrate metabolism is due to the fact that it counteracts the action of insulin in the peripheral tissues [635 a]. Compensatory increase of B-cell activity follows, and then degranulation and exhaustion of the B-cells [330]. The anti-insulin effect of prolactin does not depend on the pancreas, and is therefore not due to glucagon. It is also present in hypophysectomized and adrenalectomized animals [635-637]. The "diabetogenic" effect of prolactin is of physiological importance in that the hormone counteracts the potential hypoglycemia and depletion of glucose of the body which might otherwise occur during lactation because of the increased production of glucose within the mammary glands [635 a].

Thyrotropic hormone (TSH) also activates STH [635 b]. This effect may be mediated by the coenzyme A content of the body, coenzyme A being essential for normal carbohydrate metabolism. Thus, although in the rat STH increases the pantothenic acid and coenzyme A in the liver, and hypophysectomy reduces these substances [569], the decrease of coenzyme A in hypophysectomized animals cannot be eliminated by STH alone, but only if thyroxin is also administered. Thyroxin replaces thyrotropic hormone in the hypophysectomized animal, so that this effect is due to the fact that the TSH of the anterior pituitary is necessary for the complete action of STH on the coenzyme-A content of the liver. Prolactin also has a synergistic action in the mechanism which increases the coenzyme A content, by forming a stable complex of coenzyme A with prolactin and thus decreasing the breakdown of coenzyme A [638]. As a result of all these actions, the diabetogenic effect of the anterior pituitary represents a pathophysiological unit which cannot be appreciated by separate consideration of the actions of the individual hormones involved.

In contrast to these hormonal diabetogenic mechanisms which are located within the anterior pituitary gland, there are other, extrahypophyseal factors which affect the function of the anterior pituitary and modify its diabetogenic activity. Exogenous

cortisone can become active in this sense by reducing the production of ACTH and thus causing a paradoxical storage of insulin in diabetic patients [639]. The inhibitory effect of the sex hormones on the anterior pituitary is much more marked and results in a beneficial and outspoken antidiabetic action. In the menopause, preexisting diabetes is often improved by elimination of this inhibition of the anterior pituitary; the diabetes can again be made worse by the administration of estrogen. The beneficial effect of *estrogens* on the pancreatic diabetes of monkeys, dogs, and rats [641, 642] and on human diabetes [613] is partly due to impairment of STH production in the anterior pituitary [644]. The diabetes which accompanies acromegaly is also improved by estrogens [645].

In addition to their inhibitory effect on STH, the estrogens have another inhibitory effect on ACTH and the adrenal cortex [646, 647], as shown by the fact that castration increases cortical activity [648]. By reducing the production of corticosteroids, the estrogens can thus have an additional antidiabetic effect. This action of the estrogens is not dependent on the presence of the pancreas, but they also have a direct effect on the B-cells of the pancreas, increasing their growth and insulin content [649, 650]. The relationship between the estrogens and the islands of Langerhans is reciprocal, for human diabetes and alloxan diabetes are accompanied by disturbances in ovarian function which can be relieved by insulin [650, 651]. Ingle observed that synthetic estrogens have a diabetogenic effect in rats fed on high-carbohydrate diets, an effect which is independent of the anterior pituitary and the adrenal cortex. The significance of this finding is not known [652].

The hormones of the thyroid influence the secretion of the anterior pituitary hormones by an analogous mechanism, but the action of the thyroid hormones, in contrast to that of the estrogens, is diabetogenic. In human diabetes, the thyroid gland usually shows a 50% increase in size [256]. Hyperthyroidism and diabetes are often present in the same individual. These facts show that the increased turnover of carbohydrates induced by thyroxine may lead to diabetes ("thyroid diabetes"). As in the case of the estrogens, at least part of the effect of thyroid hormone is mediated through the pituitary gland. Since the growth-promoting and development-promoting actions of thyroxine occur by stimulation of the secretion of STH [653-655], the diabetogenic action of thyroxine is also at least partly due to this increased activity of STH. The identity of "idiopathetic" STH-diabetes and of "thyroidal" thyroxine diabetes [656] is further suggested by the observation that experimentally these two types of diabetes can be produced only in the partially pancreatectomized dog. It is impossible or extremely difficult to produce them in the rat. In addition, thyroxine diabetes, like STH diabetes, first shows a transient, reversible hyperfunction of the pancreatic islands with degranulation of the B-cells [656], while prolonged administration of thyroxine produces a "metathyroidal" diabetes with irreversible atrophy and degeneration of the B-cells and the islets. These changes are entirely analogous to those seen in STH diabetes. However, thyroid diabetes can be distinguished from STH diabetes by the presence of other etiologic factors: thus, the adrenal cortex must be involved, since thyroxine increases the production of ACTH [657] and elevates the blood level of corticosteroids [658]. In addition, thyroxine probably also acts directly on the pancreatic islands, for the increased oxidation of carbohydrates brought about by thyroxine leads to increased requirements for insulin and increased activity of the islets—ultimately, in some cases, leading to exhaustion of the islet tissue [659].

The tremendous complexity of the regulation of carbohydrate metabolism via the anterior pituitary shows that, in the pathogenesis of human diabetes, the disturb-

ances of the pituitary play an important and fundamental role – indeed, probably a central role. The fundamental nature of the diabetogenic action of the hormones of the anterior pituitary is shown not only in animal experiments but also in human diabetes. Thus, Houssay showed that hypophysectomy cured pancreatic diabetes in the dog and cat. In human diabetes, spontaneous necrosis of the pituitary or hypophysectomy causes improvement [660–664], although under certain circumstances the resulting hypoglycemia may persist and lead to death.

This analysis of the biochemistry of diabetes has been based on the fact that the fundamental defect which underlies diabetic metabolism, blocking of oxidation of glucose, constitutes a threat to the supply of energy and therefore to life itself. The status of the diabetic organism is threatened by yet another abnormality, diminution of the body protein which is the actual building material of each living cell. The effect of ACTH and the corticosteroids is to withdraw protein by gluconeogenesis, increasing the stores of sugar. The effect of the energy-requiring growth stimulated by STH is to cause an exaggerated response of the catalytic agents which liberate energy (notably, insulin), thus encouraging the development of diabetes, inhibiting the further deposition of protein and reversing previous deposition. Thus, the biochemical abnormality which constitutes diabetes involves the basic problems of energy metabolism of life, so that diabetes is a problem of the widest importance in biology.

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CHAPTER IV.

Carbohydrate Metabolism: Clinical Section

By Ferdinand Bertram

Diabetes Mellitus

In the first edition of this book, Thannhauser made a prediction: "Explanations and interpretations of the processes of metabolism which seem clear and indisputable today may be overthrown tomorrow as the result of advances in physics and chemistry." This prediction has proved to be especially true in the field of diabetes. The tremendous developments of biochemistry, the advanced studies of vitamins and hormones, and the experiences of the second world war and the post-war years of starvation have completely eliminated many of the old concepts and substituted new ones. Many problems, however, still await solution, especially in the field of the late complications of diabetes, which today in large part determine the prognosis of the individual case.

In this chapter, we shall, with Thannhauser, restrict ourselves to proven facts, and shall discuss theoretical hypotheses only to the degree that they may stimulate further investigation. The literature of diabetes has become so voluminous that only selected works can be considered in any detail.

Pathogenesis of Diabetes Mellitus

Diabetes mellitus is a chronic, hereditary disease of the entire endocrine system and the closely related autonomic nervous system. In every case, the key abnormality is dysfunction of the cells of the islets of the pancreas. All the other endocrine organs which have to do with carbohydrate metabolism are related to the pancreas by means of the blood sugar. Thus, in a broader sense, diabetes mellitus is a disease of impaired endocrine regulation. Of the organs other than the pancreas, the adrenal cortex has a special importance.

In addition to the data of clinical and experimental research, the central position of the islands of Langerhans in the pathogenesis of diabetes has recently been confirmed by the morphological studies of Ferner. According to his investigations, every type of permanent or experimental diabetes is associated with a shift of the islet-cell picture towards the glucagon-producing alpha or A-cells. In evaluating the events of metabolism, it is of no importance whether these changes represent an *absolute*

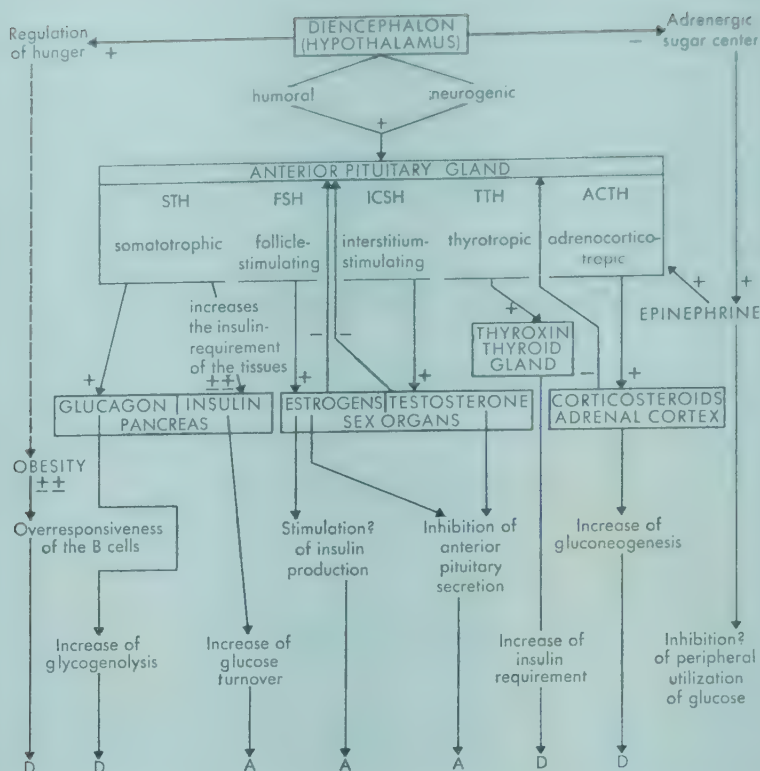


Fig. 60. The relationships among hypothalamus, anterior pituitary gland, peripheral endocrine glands, and carbohydrate metabolism.

D = diabetogenic effect A = antidiabetogenic effect

increase of the A-cells without any quantitative change in the beta or B-cells ("counter-regulation diabetes"), or whether there is only a *relative* increase of the A-cells as a result of destruction of the B-cells ("insulin-deficiency diabetes").

Of great importance in carbohydrate metabolism is the diencephalon-anterior pituitary system. The role of this system in the alimentary regulation of carbohydrate metabolism is discussed under the tests of function of the pancreatic islands (p. 264). Figure 60 is a schematic presentation of the manifold functions of this system.

The anterior pituitary produces two groups of hormones, somatotrophic and glandotrophic. The somatotrophic or growth hormone (STH), which may include other and still unknown hormones, is identical with Houssay's diabetogenic principle. STH has a direct effect on the insulin requirement and carbohydrate turnover of each body cell.

The glandotrophic hormones, in contrast to STH, exert their effects on body cells, not directly, but indirectly through other endocrine glands. With the exception of the parathyrotrophic hormone, which is still in dispute, these hormones are all related to carbohydrate metabolism. Still in doubt is the existence of a pancreotropic hormone to which is ascribed stimulation of the B-cells to produce insulin. The thyrotrophic hormone does not influence the blood sugar but causes a loss of glycogen in the liver. Thyroxine also increases the irritability of the sympathetic nervous system and in this way inhibits the effect of insulin. The gonadotrophic hormones influence carbohydrate metabolism only indirectly by dampening the activity of the anterior pituitary and thus negating counter-regulation. The adrenal cortex has been discussed in detail in the preceding chapter on the theory of diabetes. The anterior pituitary secretes ACTH which mobilizes the corticosteroids of the adrenal cortex which regulate the phosphorylation processes which are so important in carbohydrate metabolism. Some 40% of cases of acromegaly and Cushing's disease show glycosuria, but true

diabetes mellitus is found in only some 15%, presumably only in patients who already have a genetically inferior pancreas. An increased production of corticotrophic hormone and glucosteroids may be the cause for the appearance of clinical diabetes in these cases.

In tumors of the adrenal medulla (pheochromocytomas), true diabetes may rarely be present. The increased production of epinephrine in patients with such tumors may cause transient hyperglycemias and glycosurias which have nothing to do with true diabetes. In a small proportion of cases, however, the increased secretion of ACTH which is due to the increased epinephrine may result in true diabetes — again, apparently, in patients with a genetically inferior pancreas.

The incidence of diabetes rises during puberty and especially in the menopause. Menstruation and pregnancy make diabetes worse (pp. 281, 283). All these observations show the close relationship of diabetes to disturbances of various endocrine glands.

The role of the diencephalon in the etiology of diabetes has been overemphasized. It is well known that disturbances in carbohydrate metabolism are not found following operative excision of this region or after other destructive changes (injuries, tumors, hemorrhage, infection). The diencephalon-anterior pituitary is merely a center for impulses which come on the one hand from the central nervous system and on the other from the tissues of the periphery. A defect in "transmitting" or "switching" such impulses does not in itself cause disease. Disease first starts, in general, when the subordinate endocrine glands are no longer capable of compensating for the defect at the center. Such defects, thus, may be responsible for the clinical appearance of diabetes, but are never the basic cause of the disease.

The synthesis of glucose from glycogen in the liver occurs on the basis of a local, autonomous regulation which is independent of humoral and neurogenic stimulation (Soskin).

Pathogenetic Classification of the Various Forms of Diabetes

Many years ago it was customary to differentiate a "lean" and a "fat" form of diabetes. The "lean" diabetes was assumed to be due to an inherited defect of the pancreatic islets, and the "fat" diabetes was ascribed to overeating on the part of the patient. Today, it is well known that in both cases an inherited defect of the pancreatic islets plays a role. Clinically, however, one should rather distinguish two basic types of diabetes, "insulin-deficiency" diabetes and "counter-regulation" diabetes, always recognizing that there are numerous transitional forms between the two.

The chief characteristics of the two groups are listed in Table 55, which is based on an old classification given by Schmidt and Lorant. Falta has ascribed especial importance to sensitivity to insulin in this classification. In insulin-deficiency diabetes, pancreatic insufficiency alone is the key to the clinical picture; in counter-regulation diabetes, the deficiencies of other endocrine glands are also present.

Insulin-deficiency diabetes. This type is seen especially in young people and in thin, asthenic individuals; it also occurs, however, in older people. It is due to an absolute deficiency of insulin and in its clinical symptomatology simulates most closely the diabetes of the pancreatectomized animal and alloxan diabetes (p. 221). The persistence of the glucagon-producing (anti-insulin) A-cells explains why greater amounts of insulin are necessary in human insulin-deficiency diabetes than in experimental diabetes after total pancreatectomy. In alloxan diabetes, all the B-cells are destroyed

Table 55. Classification of Diabetes Mellitus

Insulin-deficiency Diabetes	Counter-regulation Diabetes
Thin, asthenic habitus	Pyknic, sthenic habitus
Usually under 30 years of age	Older age groups
Usually hypotension	Usually hypertension
Muscular weakness	Muscular, often fat
Weak, lacking in energy	Strong, energetic
Sensitive to insulin	Resistant to insulin
Tendency to ketonuria	No tendency to ketonuria
Coma frequent and typical, with good prognosis	Coma rare and atypical, with poor prognosis
Renal threshold normal or low	Renal threshold often elevated
Frequent areflexia	Reflexes normal
	Tendency to arthritis, neuralgia, calcification, gangrene
Fair, blue iris	Pituitary signs may be present

but the A-cells are not affected – a situation such as that postulated in spontaneous human diabetes of this type. The results of the deficiency of insulin are an inability to oxidize glucose and thus a progressive diminution of glycogen in the liver and muscles (p. 210). The sequelae are increasing hyperglycemia, glycosuria, acidosis, and their several consequences. All these abnormalities can be completely corrected by treatment with insulin. The good prognosis of juvenile diabetes rests on the prompt response to insulin.

Wrenshall and his coworkers showed that in juvenile diabetes there is early destruction of the B-cells with corresponding diminution of the insulin content of the pancreas, in contrast to the older patients who may not show these changes even late in the course of the disease.

Counter-regulation diabetes. This type is seen chiefly at an older age, especially at the time of genital involution, and occurs especially in persons of a pyknic, sthenic body build. The experimental prototype is the permanent diabetes produced by Young by injections of anterior pituitary hormones in animals. The concept of counter-regulation originated with Falta, who used the term for the reciprocal relationship between the islet tissue (insulin) and the adrenal medulla (epinephrine). The aim of this reciprocity, he stated, is the maintenance of a constant rate of carbohydrate utilization. The concept has now been extended to include all glands antagonistic to the islet tissue.

In counter-regulation diabetes, the basic pathogenetic event is hyperfunction of the adrenal cortex. The result is the phenomenon known as gluconeogenesis (p. 233). In contrast to insulin-deficiency diabetes, the liver is fully laden with glycogen, and large amounts of glucose pass from the full glycogen stores into the bloodstream. A normal pancreas is capable of compensating for the resulting hyperglycemia by increased production of insulin, but a genetically inferior pancreas will succumb in the long run. The hyperglycemia may result secondarily in a shift of the ratio between the A- and the B-cells in favor of the A-cells. At first, there is no absolute, but only a relative deficiency of insulin, and no anatomical, but only a functional impairment of islet tissue which may still be corrected by therapy. Later, however, irreparable damage of the B-cells can occur, so that an insulin-deficiency diabetes can develop from a counter-regulation diabetes. In parallel with the gluco-

neogenesis, other disturbances may occur which ultimately affect many tissues including the capillary system of the fundi (p. 274) and of the kidneys (p. 276), leading to the Kimmelstiel-Wilson syndrome. The features of counter-regulation diabetes thus include many signs of other disturbances. Of special importance are pituitary signs (Bartelheimer), which may be either slight or severe. Indications of disturbed pituitary function include proportional gigantism in young people, acromegaly in adults, hypertension, purple striae, x-ray changes of the sella turcica, hyperostosis frontalis interna, osteoporosis, and sometimes heterosexual characteristics (masculine growth of hair in the female, feminine distribution of hair in the male).

Disturbed function of other endocrine glands which may accompany counter-regulation diabetes include hyperthyroidism (including the full-blown picture of Graves' disease) and adrenal cortical hyperfunction with increased corticosteroids in the urine (however, the question of adrenal cortical adenoma must then be ruled out). Disturbances in the gonads may be found as an expression of the general endocrine imbalance.

Diabetes due to primary disease of the pituitary or the adrenal cortex has already been mentioned (p. 256). Bartelheimer has used the term "mesencephalic diabetes" ("diabetes due to defective regulation by the mesencephalon") for a special group of disturbances of the central nervous system. This expression is not too well chosen, since it could lead to the erroneous belief that damage of the central nervous system, specifically the midbrain, is the basic cause of diabetes.

Some authors consider insulin-deficiency diabetes and counter-regulation diabetes as pathogenetically different diseases. This possibility is unlikely, if only for the reason that they show no differences with regard to heredity. In addition, each type of diabetes ultimately shows changes in the entire endocrine system.

Some 87% of juvenile diabetics are larger than their non-diabetic, healthy contemporaries, even before the diabetes has become manifest, showing increased development of the bones and the teeth and intelligence which is above average. Joslin, Katsch, and others have assumed that these observations indicate responses to hormonal growth stimuli of pituitary origin. When the counter-regulatory mechanisms disappear, these pituitary stimuli can quickly (days to weeks) lead to damage of the B-cells and thus to insulin-deficiency diabetes. This sequence is especially likely to occur in young persons, but may also occur occasionally in older individuals. Mohnike refers to the change as a rapid "metamorphosis". It is possible, but unproven, that an especially severe hereditary inferiority of the islet cells may play a role. In the counter-regulation diabetes of the older person, the counter-regulatory mechanisms persist for a longer period of time. In this case, at the beginning, there is probably only a functional impairment of the relationship between the A- and the B-cells, and the destruction of the B-cells may not take place at all or may only occur much later. In other words, the "metamorphosis" takes place over a period of years, instead of days to weeks as in the youngster. With early and adequate treatment, it is possible in some patients to effect a reversal of the functional disturbances and in this way to cause the diabetes to remain latent. In other patients, and especially when treatment is inadequate, overt insulin-deficiency diabetes may develop even after many years.

Traumatic diabetes. The term "traumatic" should be reserved for those cases of diabetes which develop immediately after a severe injury to the pancreas. If such a form of diabetes exists, the term should be strictly applied (see below), and should never, in any case, be used for the diabetes which follows damage to the central nervous system, whether of traumatic or psychic origin. This latter form would be better classified as "neurogenic" diabetes.

Traumatic diabetes is extremely rare. It has been shown that 90% of the pancreas must be lost in order that diabetes result. Joslin, Umber, Faltz, and we ourselves have never seen a case in which upper abdominal trauma was the cause of true diabetes. Grate described a patient who developed diabetes after a severe gunshot wound of the pancreas. The diagnosis of traumatic diabetes demands satisfaction of the following criteria: (1) Before the injury, there must have been no diabetes. (2) There must be no history of diabetes in the family of the injured person. (3) The diabetes must come on immediately after the injury (weeks to a maximum of 3 months).

The literature contains descriptions of a number of cases in which diabetes was caused by severe infections of the pancreas. We ourselves have seen the onset of a diabetic state following severe acute pancreatitis; the diabetes, however, disappeared completely within several weeks in almost all cases (p. 284). If the diabetes persists, it seems probable that a genetically inferior pancreas has been damaged by the infection, and that the resulting diabetes is due only to the worsening of an already latent disorder. These questions are of great importance in compensation cases and are often difficult to answer.

Neurogenic diabetes. The existence of neurogenic diabetes is in even greater dispute. It has long been known that skull injuries, apoplexy, brain tumors, and severe psychic agitation can lead to transient glycosuria. Experimentally, glycosuria can be produced by stimulation not only of the floor of the fourth ventricle, but also of other parts of the central nervous system (hypothalamus and the upper sympathetic ganglia) as well of the peripheral nerve centers. However, the data in this regard are quite contradictory.

Even if true diabetes develops after damage to the brain, it is possible to deny a causal relationship between the two events on the basis of experience, since even severe damage to important brain centers does not lead to diabetes (p. 257). In this regard, the observations of the two world wars show no increased incidence of diabetes following injuries to the brain. The same is true for severe psychic trauma. It is time to stop talking about Strick's single dog and of Ranson's one ape (out of 50), in which diabetes followed experimental damage of the midbrain, and to point out that diabetes has not occurred in the thousands of persons with skull injuries in war and accident or in people subjected to severe psychic trauma during bombing. It cannot be emphasized enough that animal experiments permit no conclusions with regard to the pathogenesis of human diabetes, especially since household animals occasionally develop diabetes spontaneously (Ricketts et al., 1953). If diabetes manifests itself at the time of trauma, it is often necessary, from the compensation point of view, to decide the difficult question as to whether the diabetes is merely a premature manifestation of a previously latent diabetes.

Etiology of Diabetes Mellitus

Diabetes mellitus is a hereditary disease. With the exception of certain special types of diabetes (traumatic diabetes, diabetes following destruction of the pancreas), the hereditary predisposition is, in the final analysis, the basic cause of the disorder. The incidence of a predisposition to diabetes in human beings is considerably greater than is generally assumed, since not all cases become clinically manifest. It is not known whether any anatomic abnormality, in the sense of an altered relationship between the A- and the B-cells, is present in the genetically inferior pancreas. The

inheritance of diabetes is recessive, although a dominant inheritance may be simulated. Proof of hereditary predisposition can be obtained only in a limited number of diabetic patients since, often, relatives of patients with overt diabetes may die before their own latent diabetes has had a chance to become manifest. Nannyk was able to demonstrate the hereditary pattern in 18% of his cases of diabetes, and Embet in 25-37% of his cases.

If the family history can be obtained in great detail, the incidence increases. If pancreatic function tests are used in family studies, the figures also increase. Thus, Pannhies found that 65% of his diabetics demonstrated a familial incidence, and Joslin was able to prove heredity in 60% of his diabetic children who lived beyond the age of 20 years. If one identical twin develops diabetes mellitus, so does the other, but if twins are not identical, the probability that the twin of a diabetic individual develop diabetes is no greater than that of other siblings. Then Bergh reported on diabetic twins who always developed diabetes before the age of 43; in every pair, the diabetes was the same as regards severity, tendency to acidosis, complications, etc.

From the standpoint of heredity, there is no difference between insulin-deficiency diabetes and counter-regulation diabetes. There are many observations in which the first generation develops mild, senile diabetes at an advanced age, the second generation shows counter-regulation diabetes in middle age, and the third shows insulin-deficiency diabetes in a child. Lukens described these events as "anteposition" and characterized the earlier onset of diabetes in each succeeding generation as a "self-purification of nature." The ultimate result is that the disease finally occurs during prenatal life; i. e., runs its course within the uterus. This concept cannot, however, be considered as a strict rule in any sense.

It should be reemphasized that the existence of a genetically inferior pancreas does not necessarily mean that clinical diabetes will develop in every case. Diabetes, in other words, is not an inescapable fate. Thus, John observed 54 patients in whom the presence of latent diabetes was demonstrated by means of carbohydrate tolerance tests (p. 264), but only in 69% did diabetes become manifest during a follow-up period of from 1 to 25 years. Usually, in these cases, the diabetes became overt following some exogenous stimulus.

Numerous exogenous and endogenous factors may act as precipitating factors, but not as etiologic ones. Such factors include environmental influences (especially the type of nutrition), organic changes in the endocrine glands (tumors, trauma, hemorrhage), and psychic stress (p. 260).

The incidence of diabetes is increasing throughout the world, apparently for a number of reasons. Some of the "increase" is undoubtedly due to improved methods of diagnosis. In addition, people in general live longer and are thus more likely to reach the chief age at which diabetes becomes manifest (the 5th to 6th decade). Furthermore, the life span of juvenile diabetics has been increased by modern therapeutics. The major cause, however, is the changed living conditions of people. Diabetes belongs to the large group of "diseases of civilization." There is no single factor which can be blamed for this rise, but rather there are many influences to which generation after generation of people have been exposed for a long time.

As the standard of living rises, there is a change from basic rural nutrition to a more refined type of diet. The latter entails a higher consumption of animal protein and fat and a lower consumption of carbohydrates, together with lessened intake of vitamins and minerals. According to Hinshaw, the consumption of fat has risen in virtually all civilized peoples by approximately 40% in the last 2 to 3 decades. There

is no doubt that this increase plays an important role in the increased incidence of diabetes as well as its complications (p. 290). The best proof for the accuracy of this view was supplied by the starvation years in Germany, when the mortality due to diabetes fell by 40%.

Obesity itself favors the manifestation of diabetes. An increased intake of food increases the utilization of insulin and can later lead to failure of the β -cell system (p. 286, Fig. 60). Statistics show that 50-60% of all diabetics are obese.

Another important cause for the increased incidence of diabetes is the lack of physical exercise in modern life, particularly due to modern means of transportation. Diabetes mellitus is often found among persons with sedentary pursuits. In former days, diabetes was rarer in rural than in urban populations, but today this difference has disappeared. Other factors which favor the manifestation of diabetes include deficiencies of vitamins and minerals, the abuse of alcohol and other stimulants, and the misuse of certain medications. The precipitation of diabetes by pregnancy is discussed elsewhere in this book (p. 281ff.).

Whether various other events, such as infection and trauma, are capable of producing overt diabetes by their stimulus to increased production of adrenal corticosteroids - a sequence postulated by Selye - is questionable.

Racial differences have no significance. As far as the incidence of diabetes is concerned, nomadic peoples adjust to their host lands to the extent that they assume the native ways of life. Thus, for example, diabetes is rare in African Negroes, while the incidence of diabetes in United States Negroes is the same as in the indigenous population itself.

"Conjugal" diabetes does not exist. R. Schmidt thus found only 20 cases of 2320 patients with diabetes, in which both marriage partners were diabetic. The view occasionally held by laymen that diabetes is contagious is completely unfounded.

The Diagnosis of Diabetes Mellitus

The prodromal signs of diabetes include pyoderma, neuritis, pruritus, balanitis, gingivitis, furunculosis, poor healing of wounds, and impaired libido. In women, complications of pregnancy and typical changes of the fetus may precede the appearance of diabetes by years or even decades (p. 282).

The clinical signs of diabetes are the result of the disturbance of intermediate metabolism. Glycosuria is often discovered accidentally in the absence of any other signs of the disease (life insurance and other routine medical examinations). Among the first symptoms of overt diabetes is severe thirst, polydipsia. Polyuria and nocturia are frequent. The urine is clear and has a high specific gravity because of its content of glucose. The patient loses weight despite normal or even increased nutrition (polyphagia). When acidosis supervenes, severe weakness occurs. Serious symptoms may develop slowly, but sometimes may occur quite suddenly without warning and, if the diagnosis is missed, lead to death. Occasionally, diabetes presents acutely with coma, sometimes triggered by infection or pregnancy. Such cases should not be considered to have a more serious prognosis than those in which the disease develops slowly.

The diagnosis of diabetes is usually made by examination of the urine for glucose. The healthy person with normal nutrition shows only traces of carbohydrates in the urine. These are so small that they cannot be demonstrated by the usual methods. It is only after the ingestion of excessive amounts of sugar that glycosuria may some-

signs appear in healthy individuals. The minimal excretion amounts are as follows: glucose and fructose, 150 to 200 grams; lactose, 120 grams; galactose, 20 grams. The glycosuria after excessive intake of carbohydrates begins within one half to one hour and lasts for several hours.

In the diagnosis of diabetes, it is not enough to examine a single random specimen of urine. It is necessary, rather, to determine how much glucose is lost by the body (i. e., not utilized) on a standard carbohydrate intake. For this determination, measurements are made on a 24-hour collection of urine. The excretion of glucose is computed from the total volume of urine and the percentage of glucose contained therein (e. g., $1,200 \text{ cc} \times 1.2\% = 14.4 \text{ grams per 24 hours}$).

The qualitative determination of glucose in the urine depends on reduction tests, which are based on the ability of all monosaccharides to reduce certain metallic ions when in alkaline solution. These tests are not specific, however; they may be positive in sugar-free urine if the patient has taken antipyretics, salicylic acid, amidopyrine, camphor, turpentine, rhubarb, or senna. Glucose can be measured quantitatively by fermentation tests, which are seldom used in clinical practice. However, both glucose and fructose undergo fermentation. More commonly, the polarimetric method is used to measure glucose. This is based on the fact that glucose (dextrose) rotates the plane of polarized light to the right. Again, fructose (levulose), which rotates the plane to the left, can interfere with this determination.

The excretion of glucose in the urine depends on the status of the kidneys. The kidneys have a threshold for glucose: the level of blood glucose at which glucose begins to appear in the urine is called the renal threshold for glucose. In the normal individual, this value is quite constant and approximately $180 \text{ mg}\%$. In the patient with diabetes, the renal threshold can vary, and can vary at different times even in the same individual. In insulin deficiency diabetes of young persons, the renal threshold is often lowered; in older diabetics, it is often raised. The renal threshold can be altered by infections (p. 285), drugs, diseases of the kidneys, etc. In diabetic coma, the kidney can "insulate" itself against glucose as well as against the excretion of ketone bodies (p. 286).

Glycosuria may also occur without a corresponding elevation of blood sugar. This situation occasionally occurs in young, asthenic persons as the result of an anomalous decrease of the renal threshold for glucose. This condition was formerly known as "renal diabetes" or "diabetes innocens;" today, the term used is "extra-insular glycosuria," since the islet tissue is normal. It is believed to result from impaired tubular reabsorption of glucose, in which the adrenal cortex may play a role. The glycosuria is generally minimal and only occasionally moderate. In rare instances, the glycosuria can be diminished or abolished by means of adrenal cortical hormones and riboflavin.

Extra-insular glycosuria (renal glycosuria) is merely an anomaly, not a disease. The ability of the patient to function is normal. Its possible relationship to diabetes mellitus is in dispute. Joslin rejects any such relationship. However, other authors, including ourselves, believe that, rarely, transitions do occur between renal glycosuria and true diabetes mellitus. The hereditary factor also plays a role in renal glycosuria. Occasionally, both renal and diabetic glycosuria occur in the same family, and sometimes both conditions are present in the same patient.

The diagnosis of renal glycosuria is suggested by the absence of any other signs or symptoms of diabetes and is confirmed by the normal glucose tolerance test (proof of normal function of the islets) and the lack of correlation between the glycosuria

and the state of nutrition or the administration of insulin. Approximately 2% of all cases of glycosuria are extra-insular in origin. This incidence was found to increase under the psychic influences of war (Bertram, Grafe, Lawrence).

In the Anglo-Saxon literature, a distinction is sometimes made between renal and "pseudo-renal" glycosurias, and this distinction is used to explain the possibility of a transition from extra-insular glycosuria to true diabetes mellitus. We ourselves do not believe that such a distinction is justified on the basis of available data.

Renal glycosuria requires no special treatment. Nevertheless, it is necessary that the patient be under medical observation, and the ingestion of large amounts of carbohydrates should be interdicted. The appearance of acidosis does not justify the use of insulin, since such acidoses disappear spontaneously.

The prototype of renal glycosuria is the glycosuria described by von Mering in 1868 following the injection of the glycoside phlorhidzin, obtained from the rind of the roots of apple, pear, cherry, and plum trees. The blood sugar in his animals was normal or diminished. The glycosuria of pregnancy also belongs among the extra-insular glycosurias, although exact diagnosis may be difficult because of the occasional disturbances of endocrine regulation which may occur in pregnancy and which are diabetogenic in direction (p. 283).

Fructose, lactose, galactose, and pentoses can, in rare cases, be excreted in the urine as expressions of harmless metabolic anomalies of no clinical significance.

Hyperglycemia. The best single criterion for the presence of diabetes mellitus is an elevation of the blood glucose, hyperglycemia. The regulation of blood glucose and its maintenance at a constant value between 80 and 130 mg/100 ml have been discussed in the preceding chapter (p. 189). In clinical practice, the blood glucose is generally determined by a method such as that of Hagedorn and Jensen or, more recently, Frank and Kirberger, which measures the "true glucose" of the blood. The highest value reported in the literature is 2,577 mg% of glucose.

Tolerance Tests. Carbohydrate loading tests are of great importance in the clinical evaluation of diabetes. These are tests of function of the islet tissue. The simplest and most reliable are the oral glucose tolerance tests. The amount of glucose given by different investigators varies between 20 and 100 grams, and a standardized method would be of value to allow comparison of results. An intravenous glucose tolerance test may also be used, in which 20 cc of a 50% solution of glucose is given intravenously and the levels of blood glucose determined after 1, 3, 5, 10, 15, 30, and 60 minutes. The effect of 1 cc of a 1% solution of epinephrine subcutaneously on the blood glucose is also a tolerance test, but is also seldom used.

In performing these glucose tolerance tests, it is necessary to examine capillary blood, for the composition of capillary blood closely resembles that of arterial blood. In the fasting state, there is no difference between the arterial (or capillary) blood and venous blood. After the ingestion of sugar, however, venous blood contains 20 to 50% less glucose than does capillary blood. This difference between the blood glucose of capillary and venous blood is less in the diabetic, and often cannot be demonstrated at all (Fata).

If a normal individual whose fasting blood glucose has been determined is given 100 grams of glucose in a glass of water by mouth, and his blood glucose is determined after $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4, and 5 hours, a glucose tolerance curve can be drawn (Fig. 61). There is an increase of the blood glucose with a maximal value of 150 to 180 mg% after 1 hour (alimentary hyperglycemia, I). After 2 hours, the blood level has returned to the initial value because of a compensatory output of insulin (II). Since the amount

of insulin mobilized is excessive when the islet tissue is normal, the blood glucose level then falls to a level which is lower than the initial value (III). This subnormal level causes an increased secretion of epinephrine by the adrenal glands which returns the blood glucose to normal after 4 to 5 hours. Some authors hold a somewhat different view. Thus, according to Gaede, Ferner and Kastrup, the regulation of the blood glucose can be explained solely by the 2 hormones of the islet tissue. They believe that it is the hormone of the A-cells rather than epinephrine which is the real antagon-

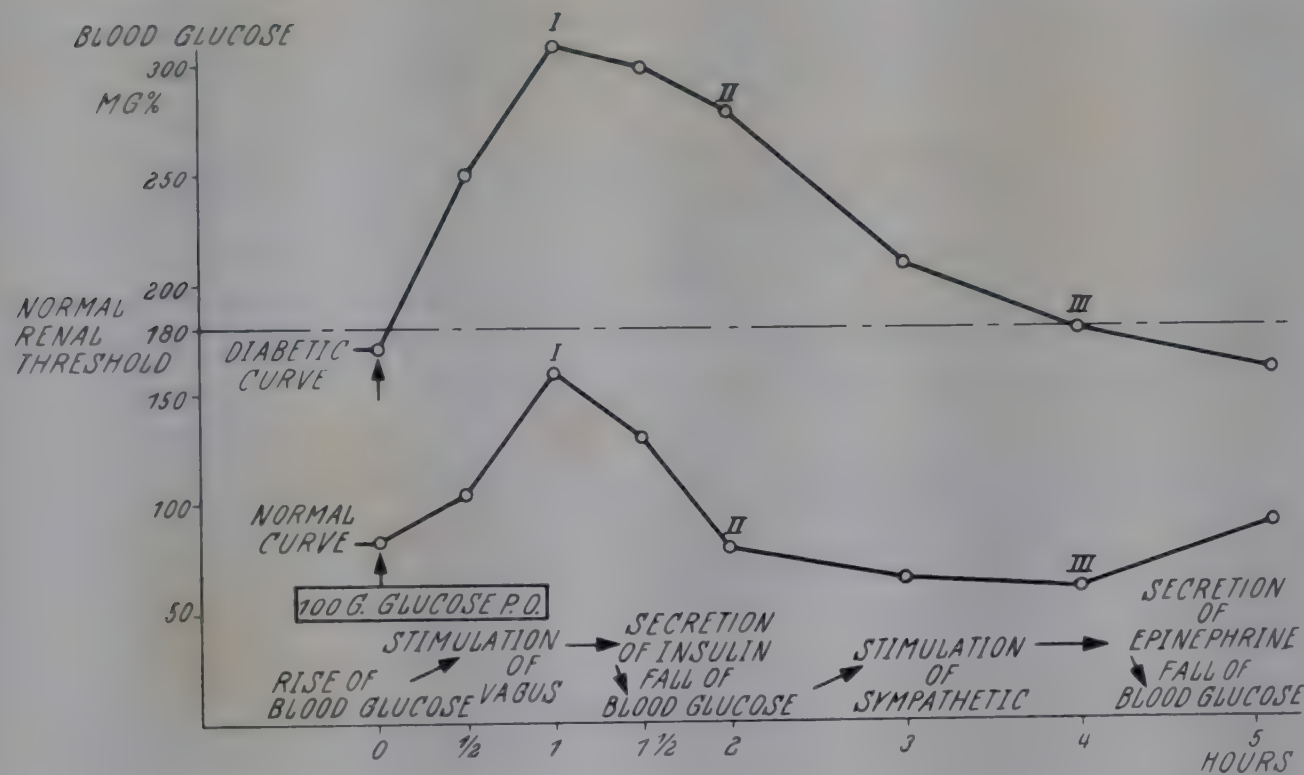


Fig. 61. The glucose tolerance curve in normal and in diabetes.

ist of insulin. This glucagon serves for fine regulation, that is, to maintain an optimal level of glucose, whereas epinephrine only serves a protective function in the sense of Cannon's emergency reaction, even without the cooperation of the anterior pituitary. At the time the blood glucose level is rising, glucagon can actually be demonstrated in the blood; as it falls, insulin appears in the blood (Bürger).

The criteria for the functional capacity of the islet tissue are the height of the rise of blood glucose (I), the rapidity of the fall (II), and the extent of the fall below the fasting level (III).

During the tolerance test, the urine is continually tested for its content of glucose. During the first 2 hours, a specimen of urine should be tested every time the blood is tested.

The glucose tolerance test of the diabetic differs from that of the normal. In the diabetic, the fasting blood sugar is often already high in accordance with the severity of the disease, and the amount of available insulin is below normal. For this reason, the blood glucose rises to much higher levels after the test dose of glucose than in the normal, and returns more slowly to the initial level. The extent of deviation from the normal is a rough measure of the severity of the metabolic disturbance. The amounts of glucose excreted in the urine are also higher, corresponding to the level of blood glucose and the renal threshold of the patient.

In general, glucose tolerance tests need be done only if the fasting blood sugar determined on several occasions does not in itself confirm the diagnosis of diabetes

mellitus. In borderline cases, the decision as to whether a given glucose tolerance curve is or is not diabetic can be extremely difficult, since deviations from the ideal norm occur even in the presence of a normal pancreas. In starvation and in patients on high-fat diets, the glucose tolerance curve shows a more rapid rise of blood glucose than normal, the maximal level attained is higher, and the return to normal is delayed. An abnormal curve may also be present in infections, in cachectic states, during pregnancy, in liver disease, and in some cases of essential hypertension. Persons of

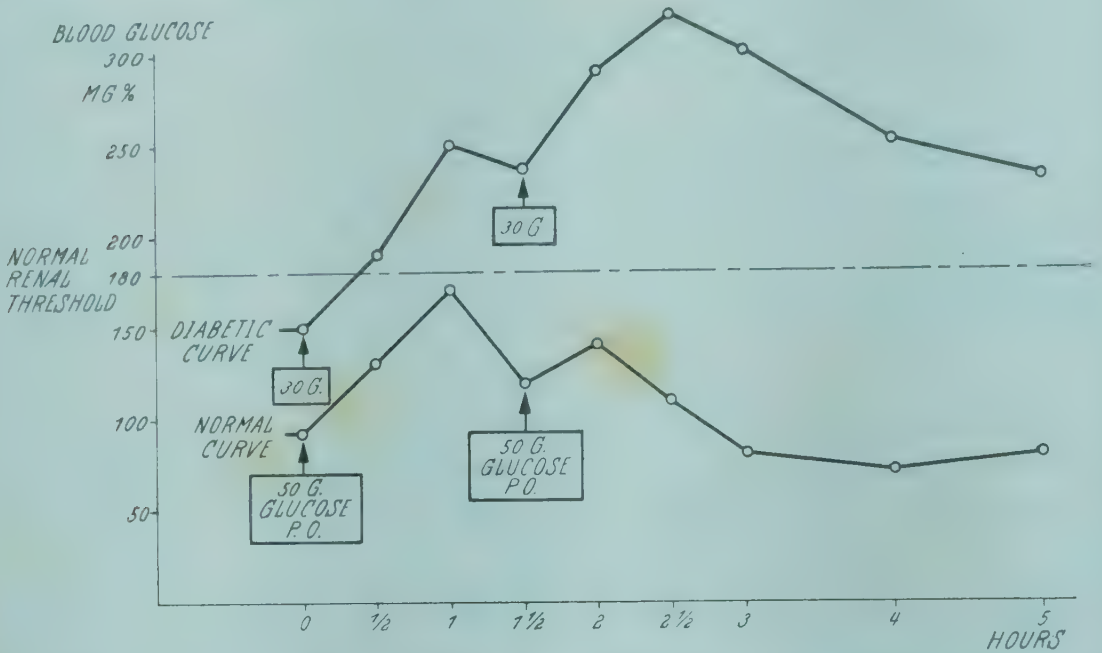


Fig. 62. The double glucose tolerance test in the normal person and in diabetes (Staub-Traugott test).

pyknic body build show rises in blood glucose which are higher and remain longer at the maximal level, and tend to a lesser hypoglycemia than the average. Asthenic persons show the opposite. With increasing age, the curves of normal individuals come to resemble those seen in mild diabetes (sclerosis of pancreatic vessels?).

An improved function test has been described by Staub and Traugott (Fig. 62), and consists basically of a double loading test. The initial dose is either 20 or 50 grams of glucose. Either at the time of the highest blood glucose (1 hour) at or the time the curve starts to fall (1 1/2 hours), the same or a larger amount is given again. With healthy islet tissue, the amount of insulin mobilized by the first dose of glucose is adequate to induce tissue utilization of the additional amount of glucose – a so-called “positive Staub effect.” In diabetics, because of the deficiency of insulin, there is a further and marked rise of the blood glucose after the second oral dose (“negative Staub effect”).

A simplified double-loading test, in which it is only necessary to determine the blood glucose three times, is used in the United States (Exton-Rose test). Fifty grams of glucose are given twice at an interval of one-half hour, and blood glucose determined initially, at one-half hour, and one-half hour after the second dose. Diabetes is present if the second peak of blood glucose is higher than the first. This method is of value only as a preliminary screening test.

In certain instances, tolerance tests using dietary carbohydrates, which are absorbed more slowly than pure glucose, have been of value. We have used a white-bread loading test for this purpose. The fasting blood sugar is taken, and the patient then receives 25 grams of white bread, 50 grams an hour later, 75 grams in 2 hours, and 100 grams three hours after the beginning of the test. The blood and urinary glucose are determined one-half or one hour after each dose of bread. The slow, repeated demands for insulin cause exhaustion of the pancreatic islands in all cases of diabetes. In the healthy person, the blood glucose curve has a slight wave-like shape and never reaches values above 160 mg^o/₁₀₀; ultimately, at the end of the test, it returns to the initial level. In the diabetic, the curve keeps rising to higher and higher levels after each dose of white bread.

Acidosis. Diabetic acidosis is due chiefly to so-called ketone bodies, which can be measured in practice by Legal's test for acetone and Gerhardt's test for acetoacetic acid. Only in severe diabetic coma, in which the kidneys can "insulate" themselves against the excretion of ketone bodies (p. 286), is it necessary to make direct chemical measurements of these substances in the blood. The normal blood values for total acetone (acetoacetic acid + acetone) are 1.3 to 2.5 mg^o/₁₀₀, and for β -hydroxybutyric acid 2.5 to 6.0 mg^o/₁₀₀.

The ketone bodies are not pathognomonic for diabetes. They occur whenever muscle glycogen is oxidized for any reason: in starvation, in a diet composed solely of fat, in the infant, in pregnancy, in fever, in intoxications, etc. The significance of the ketone bodies and the dangers of acidosis as regards diabetic coma and late diabetic complications are discussed elsewhere.

Serum proteins and lipoproteins. More and more importance is being given to serial determinations of the serum proteins and lipoproteins, especially as regards the late complications of diabetes. In general, it is believed that the blood proteins are normal in uncomplicated diabetes (Antweiler). When the disease is active and uncompensated, the total protein and the albumin of the serum are decreased. In the late complications, severer changes may occur, including reduction of the α_2 -globulins, even in the absence of liver damage (Führ and Meinecke). Lipid electrophoresis is especially valuable (p. 277). Normally, the lipids are found chiefly in the β -globulin fraction and to a lesser extent near the α -globulins. In the neglected diabetic, there is an increase of the α_1 lipoproteins. In diabetic nephropathy, there is an increase of the β -lipoproteins. In diabetic coma, all the lipoprotein fractions are increased on electrophoresis. These changes may disappear and the pattern return to normal following adequate treatment.

From a clinical point of view, cases of diabetes may be classified according to their severity. Such classification is based partly on the height of the blood sugar, partly on the amount of glucose excreted in the urine, and partly on the presence or absence of ketone bodies. Joslin and Grafe base their classification on the insulin requirement. In Joslin's clinic, a diabetes is *mild* if it requires 10 units or less of insulin per day with a diet containing approximately 200 grams of carbohydrate; it is *moderate* if 20 to 40 units of insulin are required with 200 grams of carbohydrate; and it is *severe* if more than 50 units of insulin are needed with a diet of 150 grams of carbohydrate. In Grafe's classification, diabetes is *severe* if the maintenance dose of insulin necessary to keep the urine free of glucose is over 1 unit per kilogram of body weight. In our own clinic, a case is *mild* if it remains free of ketone bodies with an adequate diet (at least 200 to 250 grams of carbohydrate daily) without the use of insulin; and it is *moderate* if it remains free of ketone bodies with diet and insulin, regardless of dosage.

The term *severe* is used only for the rare case in which it is not possible to prevent the development of acidosis in the long run.

This clinical classification no longer has any meaning as far as prognosis of diabetes is concerned. With modern therapeutics, it is possible to regulate every diabetic so that, as far as the metabolic disturbance is concerned, he is in no more danger than the normal individual. Today, the prognosis of diabetes depends largely, if not solely, on the late complications of the disease.

The Pathological Anatomy of Diabetes Mellitus

The insular system of the pancreas was first described in 1869 by Langerhans, who did not, however, recognize its true significance. The key position of the pancreas in the regulation of blood sugar, and the pathogenesis of diabetes mellitus, were first made clear by the discovery of pancreatic diabetes by von Mering and Minkowski. In recent years, histopathologic studies by Ferner, Gomori, Terbrüggen, and others have shown the existence of 2 different types of islet cells, the A (alpha) and B (beta) cells. In the normal adult, islet tissue is comprised of approximately 20% A-cells and 80% B-cells. This ratio is remarkably constant and is the same in all parts of the pancreas. The B-cells have long been known to be the site of formation of insulin. Recent investigations by Gaede and others have shown that the A-cells produce glucagon (p. 226). A change of the normal ratio between A- and B-cells always results in a disturbance of carbohydrate metabolism. In human diabetes, such a shift, with a relative increase of the A-cells, occurs regularly. This shift constitutes the specific, characteristic histopathologic basis of diabetes mellitus.

The genesis of this shift is not known (effect of the hyperglycemia? anterior pituitary effect? adrenal cortical effect?). Different species of animals show different histologic reactions. In the dog, for example, stimulation of the A-cells by anterior pituitary hormone leads to destruction of the B-cells and diabetes, whereas in the rat and the rabbit the B-cells respond with an increased production of insulin, and diabetes does not occur. In man, diabetes develops only when stimulation of the A-cells occurs in a pancreas which already has a genetically inferior B-cell system. It is also important to remember that, in contrast to the B-cells, the A-cells are found not only in the pancreas but also in extra-pancreatic tissue such as the gastric mucosa and the duodenum, where glucagon can be demonstrated (Sutherland and de Duve).

In general, the pathologic findings in human diabetes are minor and inconstant. There are no macroscopic changes in the pancreas in 50% of the cases. In the other 50%, there are atrophy and sometimes increased consistency due to an increase of interstitial connective tissue (cirrhosis). In older diabetics, fatty infiltration may be seen. Stones of the pancreatic duct and carcinoma of the pancreas are only rarely of any importance in the development of diabetes.

Histologic studies show that there is a marked variation in the number and size of the islands of Langerhans. To some extent, the variation is related to age. In the normal, healthy adult, the number of islands varies between 0.5 and 1.5 million. The total weight of the islet tissue has been calculated as between 1 and 2 grams (approximately 2 to 3 times the weight of the pituitary gland). Attempts have often been made to relate diabetes mellitus to hyaline, hydropic and vacuolar degeneration, to fibrosis, to lymphocytic infiltration, to sclerosis of the islands of Langerhans, and to arteriosclerosis of the pancreatic blood vessels. All such attempts have been unconvincing.

Warren found hyalinization of the islands in 40% of diabetics and fibrosis of the islands in 25%, but there is evidence that these changes are purely secondary in nature.

There are three pathologic findings which are most characteristic of diabetes; these are found in the kidneys, the skull, and the brain. The *diabetic kidney* was seen regularly prior to the era of insulin, but today is much rarer. The kidney is slate red over a background of fatty deposits in the epithelium of the convoluted tubules. Histologically, there is a marked deposition of glycogen in the renal tubules which gives them a characteristic transparent appearance. These deposits occur in the cells of the loops of Henle as well as the convoluted tubules. The *roof of the skull* shows a characteristic straw-yellow discoloration which is due to the accumulation of pigmented fat in the cells of the bone and the marrow. The *brain* shows increased firmness, which is probably the result of inhibition of post mortem autolysis.

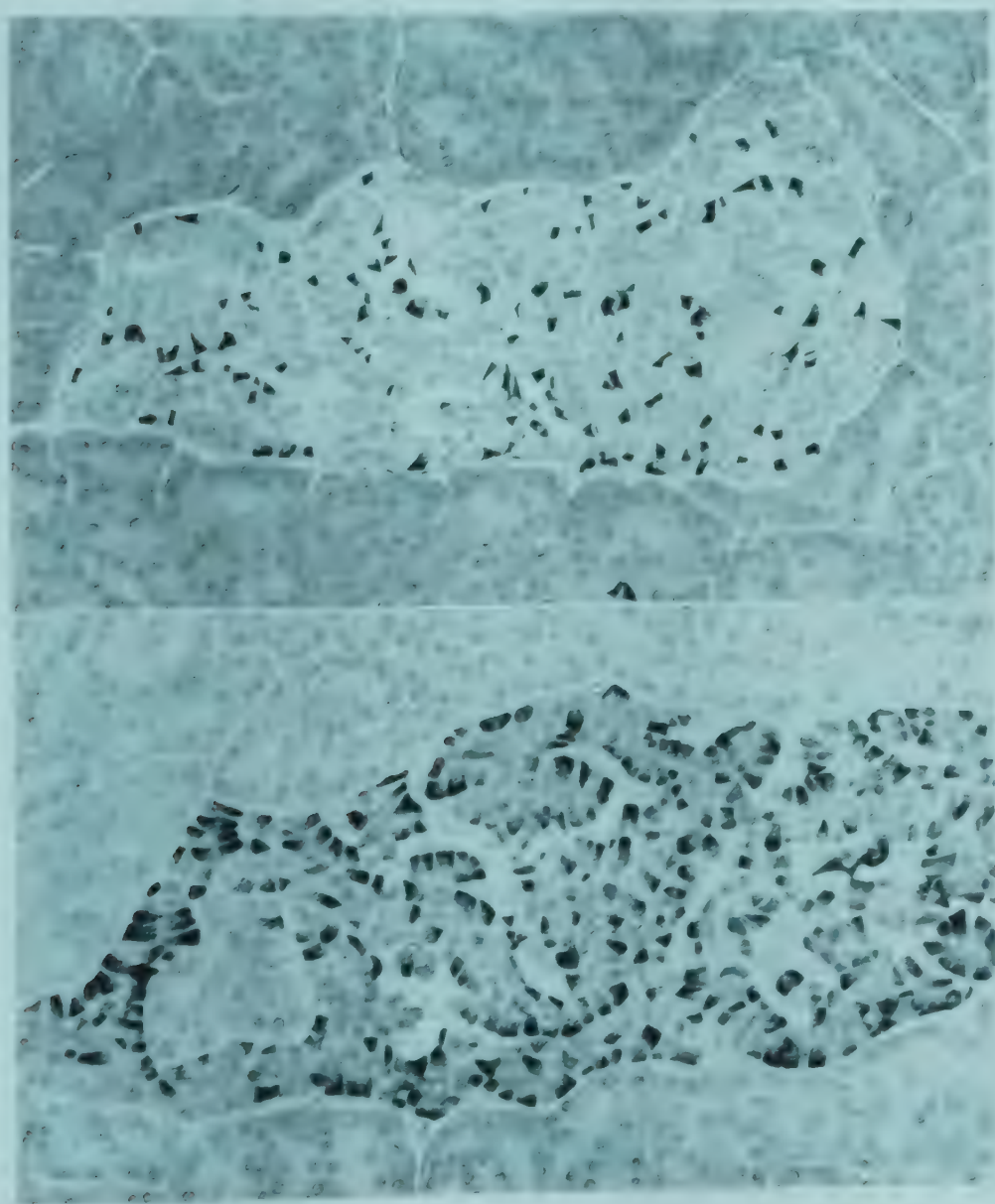


Fig. 63. The cells of the islands of Langerhans in the normal (upper picture) and in diabetes (lower picture). There is an increase of the A-cells (alpha cells), which stain black, in diabetes. 400 X. (From Ferner, *The Islet System of the Pancreas*, Stuttgart 1952.)

Other organs which sometimes show changes are the pituitary gland, the genital organs, the thyroid gland, and the midbrain, but these changes have not been shown to have any pathogenetic relationship to diabetes. In juvenile diabetes, the weight of the pituitary is reduced and atrophy of the ovaries is present.

Other Diseases and Complications in Diabetes Mellitus

It is not surprising that, in view of the widespread disturbances of total metabolism in diabetes mellitus, complications may occur in all types of body tissues and organs. It is these complications which largely determine the prognosis of diabetes. In addition, other diseases may be present in the diabetic: some of these are specifically of diabetic nature, while others are merely influenced in their course by the underlying diabetes. In former times, coma was the determining factor in the patient with diabetes; today, it is the late vascular complications.

Today, over 50% of diabetics die of disorders of the vascular system. As compared to the normal person, diabetics have unusually severe arteriosclerosis of the medium and large blood vessels, as well as disorders of the capillaries, especially in the eyes and the kidneys (Bürger's "angiopathia diabetica specifica"). It is generally accepted that the capillary changes are specific for diabetes, but such specificity is not accepted as regards the arteriosclerosis.

Arteriosclerosis in Diabetes Mellitus

The mortality statistics of arteriosclerotic diseases vary according to the conditions of life and the state of nutrition. The incidence is lowest in countries in which the diet is low in fat, and in areas of poverty (Dock, Morrison, et al.). In the United States, Germany, Sweden, etc., on the other hand, the incidence of arteriosclerosis is between 60% and 70%. The incidence is lower in Italy (Trabia and Scapellato). Knick observed marked diminution in the incidence of arteriosclerosis in the population of Leipzig during the post-war years 1945–1949: the incidence fell from the pre-war value of 69.9% to 20.7%.

Although arteriosclerosis in diabetics is partly related to age, it should nevertheless be noted that it starts earlier and shows a more serious course than in non-diabetic individuals. On autopsy, Joslin found arteriosclerotic changes in 80% of persons who had diabetes for 5 years. White and Wascow found such changes in 93% of juvenile diabetics who had the disease for more than 20 years. Our own investigations (Kuntze) have shown moderate to severe arteriosclerosis in 79.3% of diabetic men and 77% of diabetic women, as compared with 36.1% and 45.8% respectively in non-diabetic individuals.

The older view that arteriosclerosis is only due to an increase of cholesterol is not accepted today (p. 290). It is believed that the vessel wall must have undergone some damage prior to the deposition of cholesterol in the vessel. More recently, it has been suggested that there is first a deposition of an abnormal protein in the tissues of the intima, and there is then a secondary deposition of cholesterol and phosphatides. In certain cases, there may be constitutional inferiority of certain parts of the vascular system, as well as endocrine dysfunction, especially of the adrenal cortex. Diabetic arteriosclerosis primarily affects the vessels of the heart and the extremities, less often those of the brain. It is probable that an increase of the blood cholesterol in

persons who already have arteriosclerosis is harmful. Insulin deficiency causes an increased synthesis of cholesterol, and the decompensated diabetic usually shows hypercholesterolemia. In advanced cases of arteriosclerosis, Kroetz and Fischer found an increase of the β -lipoproteins and a decrease of the α_1 -lipoproteins of the serum.

Our own studies in a large number of patients (Kuntze) have shown that mortality due to heart disease is greater in patients with diabetes than in other individuals. In men, the statistical ratio was 61.5%:41.1%; in women, 65%:53%. There were no changes in the blood pressure before the fortieth year. In the older age groups, the blood pressure of diabetics showed a higher average than that of the normals. Changes in the electrocardiogram are frequent in diabetics. The chief change is one of coronary insufficiency, with depression of the ST segment in leads I and II. In addition, arrhythmia is less common than in the non-diabetic. The changes in the electrocardiogram do not depend on the severity of the disease. Many pathological changes occur together in patients with labile diabetes and a tendency to acidosis.

Myocardial Infarction. The statistics of the incidence and prognosis of myocardial infarction in diabetes mellitus vary from clinic to clinic. Joslin, Uhlenbruck, and others found that the incidence of myocardial infarction was increased; Hochrein's findings are the opposite. The mortality statistics also vary. Warren found myocardial infarction in 16.4% of 440 diabetics who came to autopsy, as compared to 4% in non-diabetics. Other observers have also stated that diabetics die 2 to 3 times as often of myocardial infarction than non-diabetics. These studies showed that the incidence of myocardial infarction in diabetes was elevated to the same degree in both sexes, while in non-diabetics the ratio of myocardial infarction in males as compared to females is 10:3. Our own studies, however, showed no difference in the incidence of myocardial infarction as compared to the non-diabetic. The prognosis, however, was considerably worse in the diabetic, the mortality being twice that in the normal. Katz also states that the mortality within the first 2 months after infarction is 50.8% in diabetics and only 26.6% in non-diabetics.

Diabetic Gangrene

Even today, gangrene is a dreaded complication of diabetes, although its mortality has decreased since 1937 from 5.1% to 1.1% (Joslin). The suggestion that gangrene is a specific diabetic change is vigorously denied by the pathologists. Bürger and others based this concept on histochemical investigations which showed a greater weight of diabetic vessels as compared with those of non-diabetics, higher values of the ash residue, and higher values of calcium and of cholesterol.

Gangrene develops most often in the lower extremities and occurs only rarely at other sites (hands, nose, ears). The precipitating factor may be found in trauma, such as tight shoes, burns, and wounds. There is no relationship between the severity of the diabetes and the occurrence of gangrene. In the beginning, differentiation from peripheral neuritis may be difficult. The dorsalis pedis pulsation may be palpable for a long time, even in severe cases. The temperature of the skin is generally reduced. The first symptoms are pain, a feeling that the part is asleep, and tingling. Dark spots of gangrene appear later. If the extremity is not treated, vesicles and infection may supervene, so that dry gangrene changes to the dread wet gangrene.

The aim of therapy is to keep the gangrenous spots dry and to permit sharp demarcation so that it becomes possible to remove a gangrenous toe without a formal operation. Each case of dry gangrene must be maintained dry, preferably with medicated powders.

In view of the inability of therapy to influence the underlying arteriosclerosis, medications and local measures which attempt to improve the circulation in the diseased extremity are largely doomed to failure. Nevertheless, we have found the paravertebral and epidural injection of novocain or a similar material to be of value. The value of vasodilators such as papaverine is in great doubt. We ourselves have had no experience with specialized massage of the extremity involved. Recently, we have also had good results with the intra-arterial injection of pyridyl-carlinol, the alcohol which corresponds to nicotinic acid. Only experience will decide whether Lemaire's insufflation of oxygen into the arteries is of any value. Local heat from a heating or infra-red lamp may be of value, but must be used with the greatest caution (p. 279). In wet gangrene, the absorption of toxic substances may be prevented by the use of ice anesthesia as recommended by Allen and his coworkers; the extremity is cooled in ice, and can remain in this state for several days without tissue damage.

Since nutritional factors are well known to play an important, sometimes decisive, role in the pathogenesis of diabetic gangrene, the patient must be placed on a very low fat diet. This diet is recommended even by Grafe, Bürger, and others who treat diabetes by means of diets high in fat and low in carbohydrates, but only after vascular damage has already appeared. We believe that the diet of the diabetic should be low in fat from the very beginning (p. 291). Beckert and certain other authors warn against the use of insulin in a patient with gangrene, claiming that it causes contraction of the blood vessels and thus further reduces the flow of blood through the affected area. In this respect, however, we agree with Bürger, who points out that it is not insulin itself which affects the vessel wall, but hypoglycemia – and this must be avoided at all costs. Insulin treatment is necessary in order to restore the normal metabolic state and thus allow adequate healing in the diseased tissue.

Gangrene can be successfully treated today by conservative means in an increasingly greater percentage of cases, but not in all cases. If the inflammatory process does not subside within a few days, operation should not be delayed too long. The old rule still holds: amputation should be made well within healthy tissue. The chief criteria which suggest that operation is necessary are a rising temperature, increasing leukocytosis, severe pains, and deterioration of the metabolic state despite energetic therapy.

Diabetic Capillary Damage

In the last few years a number of important monographs have discussed capillary damage in diabetes (Lundbaek 1953, Engleson 1954). Lundbaek uses the term "long term diabetes" for all patients in whom diabetes has been present for 15 years or longer. An increasing number of such patients show specific diffuse capillary damage in the eye grounds and in the glomeruli of the kidneys. Similar changes may be responsible for the red appearance of the skin in diabetes ("rubeosis diabetica"), but this fact is not yet decided. According to Róna, such lesions can develop anywhere in the body.

The pathogenesis of this late complication of diabetes is not settled. Virtually all authors agree that the duration of the diabetes play an important role. Some authors, such as Dolger, claim that after 15 to 25 years of the disease specific capillary damage can be demonstrated in all diabetics, but we agree with Joslin that this point of view is unnecessarily pessimistic. We have seen a large number of diabetics in whom no capillary damage could be found even after 30 to 40 years. Conversely, capillary

changes may sometimes be observed in the fundus even at the beginning of diabetes, and sometimes even in the prediabetic phase (p. 274)

It is especially important to note that the severity of the diabetes bears no relationship to the capillary changes. Other factors which play no role are the age of the diabetic, the presence of hypertension, and the presence of obesity. Of our total series, 37.6% of the women and 31.5% of the men showed specific vessel changes (Stärck). Against the once-held opinion that insulin causes or favors the appearance of capillary damage is the fact that the changes occur just as frequently in diabetics who are not treated with insulin. Neither the severity of the metabolic disturbance, as measured by the amount of insulin required, nor the height of the blood sugar plays a role in the capillary damage. Bürger believes that the degree of residual glycosuria is important in the development of the late complications. He further states that the vascular damage always develops together with an increased excretion of glucuronic acid in the urine, and concludes that there must be other "angiotoxic substances" in the body. This concept strikes us as pure hypothesis, since the increased secretion of adrenal cortical hormones is itself associated with increased excretion of glucuronic acid in the urine.

The important question as to whether the development of capillary damage depends on proper control of the diabetes is still unanswered. Wilson, Root, and Marble studied 247 patients with diabetes whose disease began before the age of 30 and lasted for over 10 years. In those patients whose disease was under complete control, the incidence of retinopathy was zero; in those with good control, 23%; in those with some control, 39%; and in those under poor control, 54%. In contrast to these findings, other experienced clinicians (Mirsky, Dolger, Mellinghoff, and others) claim that it is of little importance how well the diabetes has been managed. Thus, Dolger found retinopathy in 100% of his cases which had lasted for 25 years or more, and drew the conclusion that capillary damage is part of the picture of diabetes itself and not a complication. The definition of "control of diabetes" must be made more exact before the question can be resolved.

It is believed today that a disturbance of intermediate metabolism in the direction of acidosis must be considered important in the onset of capillary damage. This concept was confirmed in our patient studies (Stärck), although the individual groups showed certain discrepancies which could be explained by the age and sex of the patients and by the duration of their diabetes. However, we have never seen the initial manifestation or a new crop of capillary changes during acute acidosis (e. g., after an episode of coma).

There is considerable evidence that the use of a free diet favors the development of capillary damage (Engleson). Such a free diet causes marked variations of the blood glucose (Anderson) and elevation of the serum cholesterol (White).

Fanconi has made a number of important observations in this field. He found that the mortality was high in juvenile diabetics on a high-fat, low-protein, "fruit and vegetable" diet: all these patients showed kidney disease after 16 years and were dead 21 years after the onset of diabetes. In addition to the increased fat, Fanconi believed that these findings were due to a longstanding deficiency of biologically valuable proteins with resulting loss of the protective function of the liver. After this experience, he has used, since 1946, a "normal diet" which is similar to the standard diet which we have proposed. Sweets are omitted and fats are limited. Fanconi was willing to accept the presence of glycosuria. His chief criterion for adequate control was normalization of the serum cholesterol. Although the time is still too short, none

of his patients treated in this manner have as yet developed late complications, although these had been seen as early as three years after diagnosis on his previous high-fat low-protein regime.

According to some authors, patients with capillary damage show an increased incidence of damage of the liver parenchyma. Engleson found such liver damage in 40% of such patients, but we have been unable to confirm this finding. It must be recalled that even diabetics without capillary damage are subject to many exogenous and endogenous factors which favor the development of hepatic damage (p. 280). It is perfectly conceivable, however, that cirrhosis of the liver in some diabetics may rarely be a form of capillary damage.

Endocrine disturbances are fundamental in the pathogenesis of the capillary changes. In essence, these disturbances work via the anterior pituitary-adrenal cortex axis. The effect of such disturbances of hormonal control is shown, among other things, by the fact that postmenopausal women have an increased incidence of vascular damage. The first manifestations, or increased manifestations, are often seen during pregnancy. In animal experiments, the administration of diabetogenic doses of cortisone or ACTH sometimes leads to retinopathy and glomerulosclerosis (Friedenwald). It is not entirely clear whether diabetic acidosis similarly can result in the appearance or in aggravation of capillary changes, although we ourselves believe that it can (p. 273). These capillary changes which we have been discussing consist of the deposition of mucopolysaccharides, according to Altschuler and Angevine. In the final analysis, the actual development of these lesions remains unexplained. It has recently been suggested that allergy may be the key to the problem (Stärek).

Diabetic Retinopathy

Diabetic retinopathy is becoming more and more important with regard to the prognosis of diabetes. The disease was first described by Jaeger in 1855 and further detailed by Hirschberg in 1890. For some time, certain workers, such as Volhard, believed that diabetic retinopathy was the result of renal disease and hypertension, but this view is firmly denied today. Diabetic retinopathy is a disorder which is specific for diabetes and can occur without concomitant arteriosclerosis, and conversely the vessels of the fundus may show arteriosclerosis but no specific damage of the capillaries. Retinopathy is always the first sign of diabetic capillary damage (Stärek, Greig and Moro, etc.). Engleson, however, has stated that his juvenile diabetics who were treated with a free diet often developed renal disease which antedated the first changes in the eye grounds by 5 years.

Until 1937, the overall incidence of retinopathy in diabetes was given as 15%. Since 1937, this incidence has doubled. In our own clinic, Heinsius found an incidence of 32.5%, a figure which agrees with that given by Appel. The largest incidence reported is that of Bürger, who found that 46% of diabetics in Leipzig showed retinopathy, and attributed the high incidence to malnutrition in Leipzig at that time. Diabetic retinopathy affects women more often than men, and a sharp rise in incidence is seen after the menopause.

A number of stages of retinopathy may be differentiated:

- (1) Capillary micro-aneurysms and pin-point hemorrhages. Similar changes are seen in the glomeruli of the kidneys in patients with the Kimmelstiel-Wilson syndrome [313].
- (2) The above findings plus white foci of degeneration.
- (3) "Exudates" and white areas of degeneration.

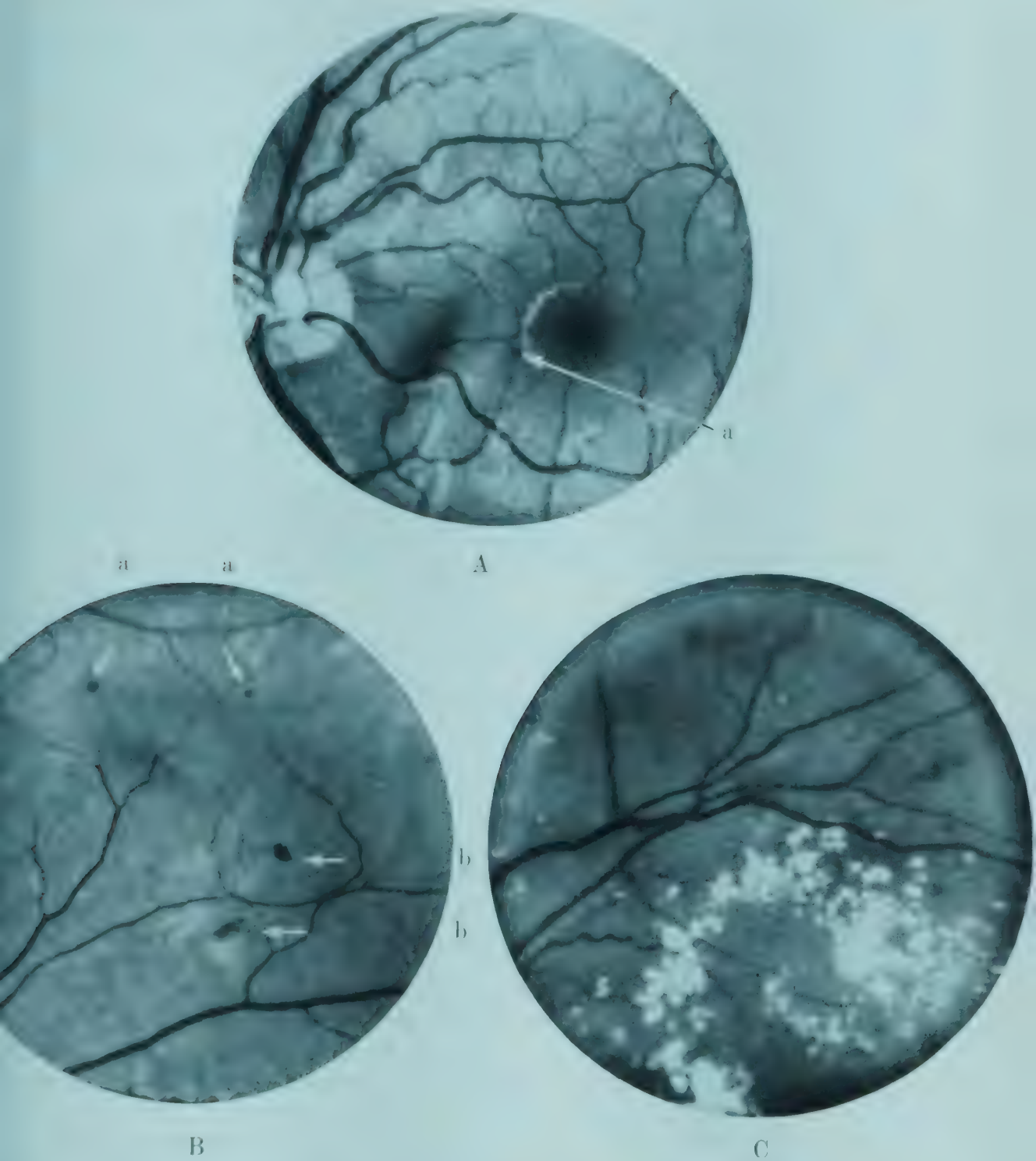


Fig. 64. Diabetic retinopathy. Courtesy of Prof. Dr. Mylius, Chief of the Eye Clinic of the General Hospital, Barmbek.

- A) First signs of diabetic retinopathy. Patient aged 19, with diabetes since the age of 6. A small petechial hemorrhage is seen at *a*, while the remainder of the fundus is still normal.
- B) Diabetic retinopathy, stage I. Patient aged 65, with diabetes since the age of 46. Small hemorrhages and microaneurysms are seen at *a*, and deeper hemorrhages at *b*.
- C) Diabetic retinopathy, stage III. Patient aged 52, with diabetes since the age of 35. Large and small white areas of retinal degeneration, some of which shine like cholesterol crystals. Among these are many small hemorrhages. Larger hemorrhages in the nerve sheaths and in the middle layers of the retina, especially marked in the periphery.

(4) Peri phlebotic processes of the type seen in severe malignant retinopathy (7 to 10% of cases). Proliferations develop from the first retinal hemorrhages. These changes are especially prominent in diabetics with a Cushing syndrome (p. 250) and in juvenile diabetics. Concomitant glomerulosclerosis has been present in all our cases of this proliferative retinopathy.

(5) Mixed forms of diabetic retinopathy and angiospastic retinitis, especially found in advanced cases of Kimmelstiel-Wilson syndrome.

Diabetic retinopathy leads to blindness in 3 to 7% of cases. Capillary resistance is often decreased, and in severe cases hypoproteinemia is often present.

Early prophylaxis and therapy are essential. Overdosage of insulin must be assiduously avoided since it can lead to acute hemorrhages. Drugs which are said to increase capillary resistance (vitamin C, rutin) are occasionally of value, but only in a few cases. A high protein diet must be given in order to prevent hypoproteinemia. Testosterone is useful in some cases (Saskin, Waldman and Pelmer, 1951; Bertram and Hemslus, 1952). The resulting improvement is probably due partly to correction of adrenal cortical dysfunction and partly to an effect on preexisting liver damage. It must be recalled, however, that spontaneous remissions are not infrequent. Wortham and Martin et al. reported reversal of capillary damage following adrenalectomy, but these studies are chiefly of theoretical interest at the present time because the extent of the operative procedure bears no relationship to the results obtained.

Other Eye Disease in Diabetes

In addition to the specific vascular changes, chemical changes can precipitate diseases of the eyes. The shrunken, soft eyeball which is characteristic of diabetic coma is the result of shifts of water in the body (p. 286), as are the transient refractive difficulties which sometimes occur at the beginning of therapy with insulin. These refractive changes have no significance, but are extremely worrisome to the patient. Improvement is spontaneous but can be hastened by the temporary restriction of salt and the use of diuretics.

Clouding of the lens may occur and must be distinguished from the usual senile cataract. The incidence of senile cataracts is greater, and their onset earlier, in diabetics than in normal individuals, but otherwise they are identical with those seen in non-diabetics. The rarer, true diabetic cataract develops in the first 40 years of life, is always bilateral, and always shows rapid progression. The prognosis of this cataract is usually quite poor. Total operative excision of the lens is recommended in order to avoid leaving behind capsule or lens residues which may cause secondary glaucoma.

Iritis is not uncommon in diabetes, and can be unusually stubborn and lead to glaucomatous changes ("rubeosis iridis").

Lipemia retinalis is a rare change which occurs as part of a generalized lipemia (p. 288). The retinal vessels resemble whitish-red or white strings. This disorder occurs only when the levels of blood fat are high.

Glomerulosclerosis

Glomerulosclerosis was first described in 1936 by Kimmelstiel and Wilson, who used the term "intercapillary glomerulosclerosis." Clinically, the syndrome is characterized by albuminuria, edema, and, in the late stages, hypertension. The histopathologic changes are so typical when they are present that they may in themselves suggest the diagnosis of diabetes at autopsy.

These stages may be distinguished within the glomeruli: fibrosis with formation of hyaline discs within the glomeruli, and hyalinization of all glomeruli. Allen showed that the hyaline material does not actually lie between the capillaries but within a thickened capillary wall, leading to occlusion of the capillaries. The original term "intercapillary" has therefore been discarded in favor of "intracapillary glomerulosclerosis." Mucopolysaccharides have been demonstrated within the hyaline

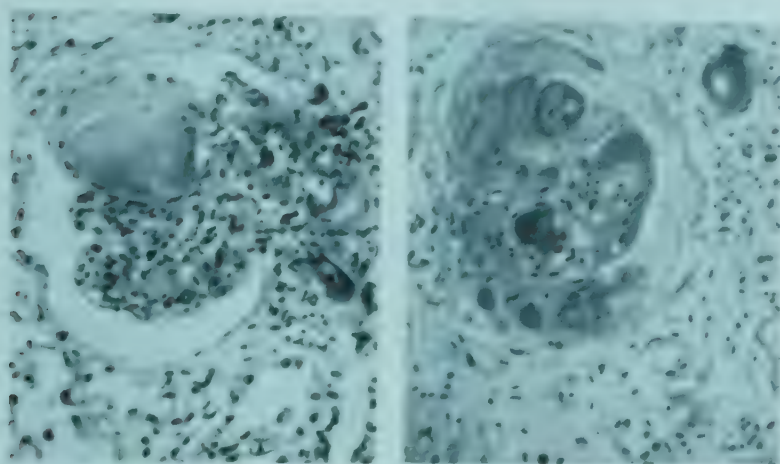


Fig. 65. Diabetic glomerulosclerosis. On the left, the glomerulus shows circumscribed nodular sclerosis. On the right, the glomerulus has become obliterated and atrophied. On the left, the tubules are still functional; on the right, they are atrophic (lower third of photograph). A sclerotic arteriole is seen above on the right. (Autopsy sections of a 71-year old woman, made by Prof. Dr. Laas, General Hospital, Heidelberg-Hamburg.)

by means of Hotchkiss' stain (Altschuler and Angevine, Becker, Allen, Ashton). These mucopolysaccharides are the chief building blocks of the intercellular ground substance, and their synthesis seems to depend on hormonal control in which the corticoids play a special role (Friedenwald). Within the blood stream, the mucopolysaccharides are bound to the serum proteins and are independent of the level of the blood sugar (McManus, Nielsen and Poulson).

Glomerulosclerosis is always accompanied by changes in the serum proteins. Electrophoresis of the serum shows reduction of albumin and increase of α_2 -globulins, a picture which is not found in diabetes without capillary damage (Friedenwald, Wuhrmann, Greff and Moro, Fuhr and Meinecke). Randerath believes that the basic defect in glomerulosclerosis consists of disturbed flow through the glomeruli of the kidneys, which results in dysproteinemia. Reabsorption of pathological proteins then causes the secondary tubular damage which is characteristic of glomerulosclerosis and which results in severe proteinuria and edema (Sarret). In some cases, aneurysmal dilatations of the capillaries, similar to those seen in diabetic retinopathy, occur.

Glomerulosclerosis is rare in non-diabetics. The moderate and severe forms occur only in diabetics. Different authors give a wide range of incidence, from 17% to 82%. The large variation is due to the fact that some authors consider glomerulosclerosis to include only the last two of the three stages named above, excluding the first stage because fibrosis itself may occur in benign nephrosclerosis, chronic pyelonephritis, and chronic glomerulonephritis. There is no correlation between the clinical picture and the histopathological findings (Róna).

A number of authors (Wilson, Root, Marble) believe that an infectious factor is important in the development of glomerulosclerosis, in contrast to retinopathy. In

actual fact, chronic pyelonephritis often accompanies the Kimmelstiel-Wilson syndrome, but the two disorders are independent of one another.

The differential diagnosis of glomerulosclerosis can be extremely difficult, and even modern kidney function tests (including clearance measurements) may not always permit precise differentiation from other chronic renal disorders (Lundback).

Two type of glomerulosclerosis may be encountered clinically. In young people and in the middle aged, a nephrotic syndrome predominates, with marked albuminuria and edema. In older age groups, the signs of nephrosis are lacking, and the patient shows hypertension without edema and without changes in the urine. Retinopathy is always present in severe cases. Approximately one-third of patients show a proliferative type of retinopathy. Approximately half the patients show increased capillary fragility.

Occasionally, especially when the diabetes is unusually labile, the development of glomerulosclerosis is associated with disappearance of the diabetes (McManus). In patients who are well, the blood sugar can rise to values which are otherwise found only in diabetic coma. In some patients hypoglycemic shock may occur after minimal doses of insulin. Acidosis never occurs in the Kimmelstiel-Wilson syndrome even in the presence of extreme hyperglycemia. It has been suggested that these phenomena are the result of decreased activity of the pituitary and prolonged overproduction of corticosteroids (Becker).

The prognosis of glomerulosclerosis is poor. Death occurs on an average of 3 $\frac{1}{2}$ years after the onset of the nephrotic syndrome, and is usually due to uremia.

Therapy is unsuccessful. The hypoproteinemia can be temporarily relieved in some cases by the use of a high-protein diet. A salt-free diet is commonly used, but has no effect. Some authors have claimed temporary amelioration of the nephrotic syndrome by the use of sex hormones, and Sarre states that estrogens have a better effect than testosterone. When uremia supervenes, the usual therapy for uremia is indicated. Luft and Olivecrona, and Kinsell and his coworkers have described reversal of capillary damage by hypophysectomy; Wortham and Martin et al., by adrenalectomy (p. 314). The dangers of such procedures generally preclude their use except in very rare cases.

Other Kidney Diseases in Diabetes

In chronic metabolic imbalance, the kidneys may show "work hypertrophy." Histologically, according to Fahr, such kidneys show enlargement of the glomerular and tubular epithelium with deposition of fat in the basal portions of the cells. Large amounts of glycogen are deposited in the cells of the loops of Henle ("glycogen nephrosis"). The renal threshold for glucose is increased (p. 263). These changes show rapid reversal with adequate therapy of the diabetes.

Pyelonephritis is common and dangerous, especially in older women. A necrotic papillitis occurs in 25% of the cases, even in juvenile diabetics, with more or less complete necrosis and a few bleeding papillary tips on pathologic examination. The complaints may begin acutely with renal colic due to occlusion of a ureter by the necrotic material. The papillae and calyces appear ragged on pyelography, the calyces showing sack-like holes at their distal portions and irregular contour of their neck.

Nocturia is frequent in untreated, decompensated diabetes, and disappears after proper therapy.

Nephrosclerotic changes are seen in older diabetics. As in glomerulosclerosis, they often lead to an increased renal threshold for glucose and in this way to what super-

usually seems to be amelioration of the diabetes - hence the need for repeated blood sugar determinations. Complete disappearance of diabetes almost always connotes glomerulosclerosis. The differential diagnosis of nephrosclerosis from glomerulosclerosis is often difficult and may be impossible.

Skin Diseases in Diabetes

Disorders of the skin are among the earliest and most common complications of diabetes. They occur in all parts of the body, sometimes with and sometimes without inflammation. Diabetes should be suspected in every case of genital pruritus (pruritus vulvae and balanitis) or pruritus in the axillae, under the breasts, or between the thighs. Diabetes should be suspected in any prolonged pyoderma, furunculosis, or carbuncle. If diabetes is present, immediate and energetic control is mandatory. Regular insulin should be used during the acute stages. The lesions themselves must be treated locally according to the usual dermatological principles. Strong and irritating medications should be avoided. The local use of heat in diabetics must be undertaken with exceptional care because of the danger of burns. Epidermiphytosis of and between the toes can be especially troublesome and can sometimes lead to gangrene. In patients in coma, special care must be used to avoid the development of decubitus ulcers (change of position, skin massage). In diabetes in general, the mucous membranes as well as the skin are easily injured, a fact which should be kept in mind in intubation, catheterization, and cystoscopy.

Today, certain skin changes which were formerly favored by the vegetable and high-fat diets once recommended for diabetes, have become uncommon. These include diabetic xanthosis (von Noorden) and xanthomatosis. Xanthosis is a yellow discoloration of the skin, especially the palms, which is due to the deposition of carotene (xanthophyll) derived from vegetables. Xanthomatosis is the development of yellow-red nodules in association with the lipemia of diabetes.

Von Noorden used the term "rubeosis diabetica" for a rosy color of the forehead, cheeks, hands, and feet seen in some patients with diabetes. Some authors attribute this condition to capillary damage due to decreased capillary tonus (p. 272).

"Necrobiosis lipoidica diabetorum" is a rare skin disease which occurs chiefly in young girls and which may precede the clinical appearance of diabetes itself. Initially, there are reddish papules which are found chiefly on the extremities and enlarge to yellow plaques as large as 2 centimeters. It is not known whether vascular disturbances (endarteritis) or lipid disturbances are of importance in the development of these lesions.

Deficiency of the vitamin B complex may lead to rhagades at the corners of the mouth, atrophic lingual mucosa, and dysphagia (Plummer-Vinson syndrome). Rapid healing occurs with the administration of large amounts of the B vitamins.

Diseases of the Nerves in Diabetes Mellitus

One of the most troublesome and severe complications of diabetes is diabetic neuritis, a late complication and one often found in association with retinopathy and glomerulosclerosis. There is disturbed catabolism of carbohydrates in the nerves, perhaps on the basis of a vitamin B deficiency (p. 290). All nerves may be affected. Diabetic neuritis is extremely resistant to therapy. The diabetes itself must be under optimal control. Large doses of vitamin B₁₂ (e. g., 1,000 μ g intramuscularly daily)

sometimes seem to cause improvement, and sometimes diathermy may be of value. In severe cases, symptoms persist despite therapy, and symptomatic sedation becomes necessary.

Absent tendon reflexes are rather common in juvenile diabetes, and posterior column symptoms may be present. Such changes are due to involvement of the long spinal tracts.

Trophic changes may appear in the teeth, nails, and hair.

Diseases of the Digestive System in Diabetes Mellitus

Caries, gingivitis, pyorrhea, and periodontal disease are common in diabetes. Granulomas of the roots may appear and aggravate the diabetes. Proper dental and gingival care are correspondingly important in patients with diabetes.

Bilateral enlargement of the parotid glands may occur in diabetes. Mellinghoff considers this a sign of "extra-insular endocrine dysfunction."

Diseases of the stomach, and especially peptic ulcer of the stomach and duodenum, are less common in diabetes than in the normal individual. Gastric hypo- and anacidity, however, are more common, and may be associated with diarrhea and, like prolonged vomiting, may lead to severe dehydration, hypochloremia, and coma (p. 286). Patients with diabetic neuropathy occasionally show night diarrhea with 10 to 15 brown, watery stools, even in the absence of achlorhydria. Vitamin B₁₂ injections sometimes promptly eliminate this symptom. Infectious diarrheas, as in the non-diabetic, respond to appropriate antibiotic therapy and replacement therapy, including clyses of physiological saline as necessary.

The Liver. Changes in the liver, which is the chief organ of glycogen metabolism, occur more frequently in the diabetic than in the normal individual. The concept of a special "hepatic diabetes", described by Naunyn and other authors, has been discarded. In general, abnormal liver function tests occur more frequently in diabetics than in non-diabetics.

Fatty Liver. When diabetes is not controlled, the liver loses glycogen and deposits increased amounts of fat. Urobilinogenuria is almost always present. Mauriac's syndrome includes enlargement of the liver because of deposition of both glycogen and fat in the juvenile diabetic (p. 316).

Hepatitis. The incidence of hepatitis is increased in diabetes, and hepatitis may be endemic. Some of the cases are certainly serum hepatitis transmitted by infected needles (not insulin needles, which are used by only one person, but needles used in blood counting, probably). The prognosis of a hepatitis must always be guarded because of the danger of transformation into cirrhosis, which is greater than in non-diabetics. Diabetes often becomes worse during hepatitis, but sometimes it shows improvement, the reasons being unknown. Therapy of the hepatitis follows the same basic principles as in the non-diabetic with the exception that pure sweets must be avoided. Duodenal washing with glucose, even when large amounts of glucose are used, does not aggravate the diabetes. In the acute stages, regular insulin should be used in divided doses, and shock must be avoided at all costs.

Cirrhosis. The older authors gave an incidence of cirrhosis in diabetes of 0.25% to 1%, but there is no doubt that the incidence has increased in recent years. In many cases, cirrhosis is not suspected until autopsy.

Bronzed diabetes is of special importance, for this may represent one of the rare forms of diabetes in which there is no hereditary inferiority of the islands of Langer-

trans. According to Lawrence, capillary damage is almost never present. Bronzed diabetes is part of the syndrome of hemochromatosis, and is usually benign. Acidosis almost never occurs. Some cases are markedly resistant to insulin. Thus, Joslin reports a patient who required 1,680 units of insulin daily. However, other cases are hypersensitive to insulin. The reasons for these differences are unknown.

The Bile Passages. Disorders of the biliary tract are found in 25% of patients with diabetes. The suggestion has been made that biliary infections might be of etiologic importance in diabetes. In such cases, Katsch considered the diabetes a "second disease" due to ascending infection of the islet tissue, but this concept has been abandoned today. There are no contraindications to the use of oral or intravenous dyes for the visualization of the gall bladder on x-ray.

The Pancreas. Acute pancreatitis is often associated with disturbances of carbohydrate metabolism which may progress to a true diabetes requiring insulin. In general, however, the picture completely reverses itself and the diabetes disappears in a matter of days or weeks. The question of whether pancreatitis can produce diabetes in the absence of genetic inferiority of the islet tissue is still in dispute. Some authors, such as Nothmann, claim an increased coincidence of chronic pancreatitis and diabetes (25%). We have no experience in this regard. If one considers that one-tenth of the pancreas is enough to prevent the appearance of diabetes, one must deduce that many of these cases must have had a preexisting tendency to diabetes. A relationship between pancreatic damage and diabetes can be accepted only rarely (p. 260).

There is considerable recent experience on total extirpation of the pancreas (for malignant tumor) in diabetes. The requirement for insulin was appreciably lower following the operation than prior to the operation. The difference is explained by the extirpation of the glucagon-producing (anti-insulin) A-cells (p. 257).

Disturbances of the exocrine function of the pancreas are not associated with the picture of diabetes.

Pregnancy and Diabetes Mellitus

Diabetic men may develop early impotence, and diabetic women amenorrhea. Juvenile diabetics show cryptorchidism more frequently than non diabetics. During the menstrual cycle, marked changes in glucose tolerance and even coma may appear in diabetics. The chief time of appearance of diabetes is the climacteric, but puberty also shows an increased incidence of diabetes.

Pregnancy constitutes a special burden for diabetic women. Even in the absence of diabetes, 8% of pregnant women show persistent elevation of the blood sugar 2 hours after the test dose in the glucose tolerance test. Before the advent of insulin, pregnancy was a rarity in diabetic women, but today sterility is rare in diabetics. Abortions are only slightly more frequent than in non-diabetic women. Maternal mortality in diabetics, formerly 30 to 50%, is now 0 to 4.1%, but neonatal mortality is unchanged at 30 to 40%. (This figure includes stillbirths but excludes abortions.) White has recently reduced neonatal mortality to 11% by the use of hormones. In her series of patients, approximately 50% of the deaths occurred by intrauterine death in the third trimester of pregnancy, 25% occurred during parturition, and 25 to 30% in the first 24 hours postpartum or as late as 14 days after birth (Given).

Congenital defects are found in 6.3% of children born of mothers with diabetes, as compared with 0.9% in the normal mother (Peel and Oakley). Intrauterine death was formerly explained by the diabetic disturbance of metabolism, but other factors are

believed to be responsible today. The duration of the diabetes is important, especially if vascular complications have already occurred. Calcification of the pelvic arteries is a particularly unfavorable sign. White states that the chance of a living child is only 20% if diabetes has been present for over 20 years.

The high fetal mortality and the increased incidence of toxemias of pregnancy, with hypertension, albuminuria, changes of the fundus, and eclampsia - 40% as compared to 7% in non-diabetic women - are caused, according to Smith and Smith (1934) by a "hormonal imbalance." In the normal woman, there is a steady increase of progesterone to a maximum in the 38th week of pregnancy. Estrogen also rises, but only in the last trimester. Later, there is a rapid fall in these levels. In women who have toxemias of pregnancy and intrauterine death of the fetus, in contrast, there is a fall of the serum level of estrogen, a reduced excretion of progesterone (measured as the pregnandiol content of the urine), and a rise of the serum level of chorionic gonadotropic hormone. These abnormal findings are present in a large percentage of pregnant diabetic women. In such women, White found that only 58% of the children survived, while in diabetic women with normal endocrine balance 95% of the children survived.

Children born of diabetic mothers often show increased size and obesity; in extreme cases, the newborn baby may weigh as much as 7,000 grams. The increase is chiefly due to large deposits of fat and to edema. Martius used the term "diabetic giant" when the birth weight was 4,000 to 5,000 grams. Enlargement of the body organs - heart, liver, spleen, and especially the pancreas - is described. The "stuffing" of the fetus was formerly explained by hyperglycemia of the mother, but against this explanation is the fact that diabetic mothers whose blood sugar is almost normal also bear large children, and also the observation that giant children have been born when the mother was still pre-diabetic. The current explanation is that the increased growth is due to stimulation of the adrenal cortex by the ACTH of the anterior pituitary (Hoet). Even this explanation, however, has been disputed. Jackson has shown that children of diabetic fathers and normal mothers also have an increased birth weight. This observation indicates that these large children are not produced solely by maternal factors and that genetic factors also play a role.

Another explanation is suggested by the increased amount of insulin in the pancreas of diabetic children. Salter and Best regard insulin as a growth hormone.

In 1928, Bowcock and Greene pointed out that all the complications of pregnancy which occur in diabetic women can often be detected years or even decades before the appearance of the diabetes itself. Van Beek, Oakley, Hoet, Kade and Dietel, and other workers have recently studied these problems of the diabetic phase. There is only a slight difference between the fate of children born of pre-diabetic mothers and those with overt diabetes. The mortality of children born in the pre-diabetic phase is increased and is given in the literature as 20 to 40%. The nearer the birth to the time of appearance of the diabetes in the mother, the higher the mortality. Prediabetic mothers show an increased incidence of both stillbirths and premature births, as well as overweight children. Thus, for practical purposes, women who show an increased incidence of abortion, premature births, or stillbirths may later develop overt diabetes especially if they belong to diabetic families. Prophylactic measures, such as avoidance of obesity, use of a milk-vegetable diet, and a careful way of life, might possibly prevent the appearance of diabetes.

Postnatal mortality was formerly explained chiefly by hypoglycemia. However, even newborn children of non-diabetic mothers may show severe degrees of hypoglycemia without clinical symptoms (p. 319), so that the truth of this explanation is in question.

Diabetes may first appear during pregnancy. It may then persist or, alternatively, may again become latent after delivery, especially in primiparas. Sometimes diabetes may begin during pregnancy as coma. Such coma does not necessarily connote a bad prognosis, since proper treatment may allow even such cases to become latent after pregnancy. If diabetes preexists, pregnancy aggravates the diabetes in 50% of the cases, and improves the diabetes in 10% (Kade and Dietel). Amelioration of diabetes during pregnancy was formerly explained by the passage of fetal insulin across the placenta to the mother. Today's explanation is inhibition of the secretion of diabetogenic hormone of the anterior pituitary by estrogens (p. 256). This concept is supported by the observations of White and others that the use of estrogens not only reduces neonatal mortality but at the same time improves the diabetes of the mother.

When glycosuria occurs during pregnancy, one must distinguish between the so-called glycosuria of pregnancy, which belongs to the group of extra-insular (renal) glycosurias, and true diabetes. Glycosuria of pregnancy is generally considered harmless, but is not always so. During the first pregnancy, it can have the characteristics of renal glycosuria, but in further pregnancies it may show the beginnings of true diabetes mellitus. The glycosuria of pregnancy can thus be an expression of a pre-diabetic phase, so that one must be prepared for the birth of a giant child.

Therapy must be approached from many points of view. Careful metabolic control by an internist is essential. The diet must be rich in carbohydrates and poor in fats, in order to counteract acetonuria of pregnancy. It must contain adequate amounts of all vitamins, especially vitamins B and C, and of minerals, especially calcium. Salt should be restricted after the sixth month. Except for the mildest cases, insulin is necessary to prevent diabetic embryopathies. The blood sugar should be kept normal and constant, but shock must be prevented. During parturition, small doses of regular insulin should be used. Postpartum, the insulin dosage must be reduced rapidly since the insulin requirement falls and severe hypoglycemia can develop.

The time and type of delivery are not agreed upon by all authors. In most cases, premature delivery in the 36th to 38th week seems best. Toxemia, hydramnios, and the development of giant children usually take place at this time. Birth should always take place in a hospital. The question of vaginal vs. abdominal birth is disputed. Caesarian section is advocated in primiparas, in diabetics with previous stillbirths or premature children, in diabetics with a history of giant children, in the presence of capillary damage, and in the presence of pelvic vessel calcification. In the latter complications, section is recommended before the 36th week. X-ray estimates of the size of child and pelvis may be made in selected cases.

The results of hormone therapy introduced by White on the basis of the studies of Smith and Smith are impressive (Table 56). Neonatal mortality was reduced to 11%. Caesarian section was performed in 70% of the cases. Pedersen obtained the same results by adequate control of the diabetes without hormone therapy and without Caesarian section. Other authors, however, continued to find a mortality of 25%, but it must be noted that they had fewer cases and often used lower doses of hormones. White, Korby and Duckers divided their diabetics into 6 groups according to the duration of diabetes and the type of complications and correlated the time of delivery and hormone therapy with this classification.

With regard to neonatal care, giant children should be treated in the same way as premature births. They are very susceptible to infections and to asphyxia, and so require the prompt administration of oxygen and antibiotics. The use of glucose is

Table 56. Hormonal Therapy of the Pregnant Diabetic Woman
(from P. White, 1953)

Description	Groups					
	A	B	C	D	E	F
Duration of diabetes	very short	up to 10 years	10-19 years	over 20 years	no relationship to duration or age at onset	
Onset of diabetes	—	after age 20 years	age 10 to 20	before age 10		
Vascular complications	none	none	none	retinopathy; calcification of arteries of legs	calcification of art. of legs	chronic renal disease
Therapy						
Dose of stilbestrol and progesterone, mg/day of each, according to week of pregnancy						
-16th	0	25		25	25	
17th-19th	0	25		50	50	
20th-23rd	0	50		100	100	
24th-29th	0	100		125	125	
30th-33rd	0	100		150	150	
34th-last	0	125		200	200-250	
Time of delivery	normal	38th week			35th week	
Cost from 16th week on	none	approx. \$ 1,600		approx. \$ 2,800	approx. \$ 3,000	

always indicated, even without hypoglycemic symptoms: 5 to 10 cc of a 5 or 10% solution of glucose are given every 2 to 4 hours until the infant can take food.

With regard to therapeutic abortion, it was formerly held that diabetes as such was no indication for such a procedure. Recently, however, many authors have pointed out that the late complications of diabetes can develop or become worse during pregnancy (Beetham et al.). With preexisting retinopathy and glomerulosclerosis, pregnancy should be interrupted. Therapeutic abortion is also indicated if x-rays show arteriosclerotic changes in the vessels of the pelvis and the lower extremities. After 3 or more Caesarian sections, or in the presence of late diabetic complications, sterilization is indicated.

With modern methods of therapy, there is no contraindication to breast feeding of the infant.

Diseases of the Thyroid and Diabetes Mellitus

Disturbances of the thyroid are rare in diabetes mellitus (1-2.1%). Three percent of patients with hyperthyroidism have diabetes mellitus. A high percentage of patients with hyperthyroidism (30%) show glycosuria with normal pancreatic islet tissue. The marked increase of carbohydrate oxidation causes a depletion of liver glycogen. The absorption of glucose is increased because of the increased phosphorylation. The liver loses the ability to convert and store glucose and fructose (Kugelman).

Infections and Diabetes Mellitus

Infections of all sorts are among the most dreaded complications of diabetes because they almost always cause deterioration of the diabetes, probably by (1) damage to the pancreas and the liver; (2) stimulation of the pituitary-adrenal cortical system in the sense of a stress reaction; (3) acidosis; and (4) tryptic enzymes of the increased leukocytes. Occasionally, infections bring latent diabetes out into the open. Infectious diseases are potentially more serious in diabetics than in normal individuals because of decreased vitality, dysproteinemia, diminished production of antibodies, and the presence of acidosis. Fever can increase the renal threshold for glucose, so that determinations of the blood glucose are necessary for proper management of the diabetes. The purpose of treatment is to prevent deterioration of the metabolic situation, and the amounts of insulin required to attain this goal may be very large. Since depot forms of insulin often take too long to work, the use of frequent doses of regular insulin is recommended during acute infections (p. 298). The diet should be rich in carbohydrates and low in fats.

In rare cases, infection may cause amelioration or even complete subsidence of diabetes. This phenomenon has long been noted in cases of tuberculosis, and usually occurs when a mild case changes to severe exudative tuberculosis. The improvement cannot be explained solely by the anorexia which is often present in the cases. Lundberg believed that insulin-like substances are produced in the tissues of patients with pulmonary tuberculosis, but this concept is contradicted by the occurrence of similar improvement in other disease processes associated with purulent degeneration (pneumonias, carbuncles, necrotic carcinomas). Malaria, however, may show the same changes. The effect is probably due to damage of the liver (see spontaneous hypoglycemia, p. 319), but may also be due to changes of central regulation.

The incidence of tuberculosis is increased in diabetics, especially in young diabetics, being 12 times as frequent as in non-diabetics (Joslin). Tuberculosis is especially prone to occur after severe diabetic coma. In 80% of cases, diabetes precedes tuberculosis by a matter of years. When diabetes is not under control, tuberculosis shows a tendency to rapid exudation and caseous necrosis (Burkhardt). There is often a discrepancy between the clinical findings and the X-ray picture: i. e., extensive x-ray changes may be present with few or no physical findings. Regular x-ray studies are therefore indicated, especially in diabetics who become and remain worse after prolonged coma. Pulmonary tuberculosis is common in diabetes, but extrapulmonary involvement is quite rare.

The prognosis of tuberculosis in diabetes has improved considerably. Successful therapy requires optimal regulation of the diabetes, employing the same principles as in non-tuberculous diabetics. Overfeeding of fat must always be avoided. Initially, large amounts of insulin may be necessary, and during fever it is best to use regular insulin. The diabetes may be unusually labile, especially initially. When the diabetes is successfully controlled, the tuberculosis can be treated with the same degree of success as in non-diabetic individuals by anti-tuberculosis drugs and surgery when indicated (Vieth). Hospitals or special sanatoria are best for such patients, where appropriate specialists can work together in the treatment of the patient.

Syphilis, which was once believed to produce some cases of diabetes by involving the pancreas, is only rarely of any importance in the pathogenesis of the disorder. We have, however, seen occasional cases in which anti-luetic therapy caused improvement or even complete regression of longstanding diabetes.

Malignant Tumors and Diabetes Mellitus

With the exception of carcinoma of the pancreas, the incidence of malignant tumors is no greater in diabetics than in patients without diabetes. Marble states that 2.6% of diabetics show malignancies, of which 13% are carcinoma of the pancreas. McKitt-
rick gives an incidence for pancreatic carcinoma of 32.4% in diabetics and 2.5 to 4.8% in non-diabetics. Pancreatic carcinoma is not, however, a cause of diabetes. The mortality from carcinoma in diabetics has risen from 1.5% to 8.9% in the past 50 years (Joslin), an increase which can be related to the longer life span of the diabetic.

Diabetic Coma

Diabetic coma is the extreme, most severe stage of the diabetic disturbance of metabolism, "the increase of acidosis to the point that it immediately threatens or destroys life, clinically a severe intoxication of the body" (Kussmaul, 1874). The mortality of diabetic coma, which was 64% in 1900 (Joslin), has fallen to a level of 15 to 20%, and some clinicians give even lower figures. Joslin reports that not a single one of his last 150 patients in coma died.

Diabetic coma constitutes the extreme of the metabolic abnormality known as diabetes and is associated with loss of consciousness. The alkali reserve falls to values below 20 volumes % (9.1 meq/l). The blood glucose is always elevated. Examination of the glucose excreted in the urine is unreliable because the renal threshold for glucose is often raised in coma. The same is true for ketonuria, which may be absent even in the most severe cases.

In the stage immediately preceding actual coma ("pre-coma"), the sensorium is clouded and the alkali reserve is already considerably decreased, but the patient still responds to stimuli and is oriented as to time and place.

Cases in which coma and death are due, not to the diabetes itself, but to complicating diseases, should not be called coma, and there is no virtue to Naunyn's term "atypical diabetic coma" for such cases.

The severity of the process is determined by an accumulation of end products of carbohydrate, fat, and protein metabolism. The height of the blood glucose plays a lesser role in the coma, but acidosis is of prime importance. Cases of coma without acidosis must be viewed with skepticism and the diagnosis questioned. The ketone bodies were shown by von Noorden to be of particular importance in diabetic coma. Total acetone and β -hydroxy-butyric acid in the blood can reach values of over 100 mg% and 200 mg% respectively, and Magnus-Levy described the excretion in the urine of up to 100 grams of ketone bodies per day. The clinical picture of diabetic coma parallels the height of the ketone bodies in the blood only to a certain extent, which is partly related to the ability of the body to become used to the abnormal substances. Thus, if carbohydrate regulation fails suddenly (for example, if insulin is withheld), the ketone bodies can increase suddenly and cause severe coma even in the presence of relatively low values. If acidosis develops slowly, however, high levels of ketone bodies can be reached without coma.

The clinical picture is also influenced by the accumulation in the blood of substances which normally pass out in the urine. Thus, two-thirds of patients in coma and one-third of pre-comatous patients show an increased non-protein nitrogen and a decreased chloride in the blood. It is no longer believed that the increased NPN is the result of kidney damage, but that disturbances of water and salt metabolism are

responsible. Meyer-Bisch showed that hyponatremia may be the first sign of beginning coma, and the depletion of chloride leads to an increase of nitrogenous substances in the blood. The result is a picture of "uremia due to deficiency of sodium chloride." These disturbances of salt and water metabolism are closely associated with the adrenal cortex (p. 308).

Coma can develop suddenly within a matter of hours and without previous warning. Usually, however, coma develops over the course of hours to several days, and has definite prodromal signs which include anorexia, muscular weakness, and drowsiness. Coma may be precipitated by muscular effort, psychic stress, infections, gastrointestinal disturbances, pregnancy, and menstruation. In many cases, no such factors can be found, especially when the disease itself tends towards acidosis because of improper diet or insufficient insulin. In the final analysis, diabetic coma is always a **state of extreme deficiency of insulin.**

The prognosis of diabetic coma depends on its intensity and its duration. If therapy is started early, complete regression of all manifestations may occur. However, if coma is profound and of long duration, irreparable tissue damage to vital organs may occur which can lead to death even after the acidosis is controlled. Circulatory and renal damage are of greatest importance in this regard.

The classical description of diabetic coma was given by Kussmaul. Attempts have been made to differentiate "dyspneic coma" from "cardiovascular coma" and "renal coma," but the clinical pictures overlap. Circulatory damage is especially severe in the asthenic juvenile diabetic, but the other symptoms are also present: clouding of the sensorium, Kussmaul respiration, fruity acetone odor to the breath, general dehydration. The eyeballs are soft and sunken. The blood glucose is elevated, generally to values between 400 and 800 mg%. Glycosuria is often diminished because of an increased renal threshold for glucose and may even, in rare cases, be absent. Increased ketonemia is pathognomonic for coma and is always present, but ketonuria may be absent for the same reason as for glycosuria. The alkali reserve of the plasma is lowered. The NPN and BUN are increased. In severe acidosis, the electrocardiogram shows changes associated with hypopotassemia: depression of the ST segment, increased QT time, flattened or inverted T waves (p. 308). The blood shows leukocytosis. Albuminuria, up to a value of 0.4%, is almost always present. The urinary sediment often shows the "coma casts" first described by Külz, which may sometimes be seen before the development of coma itself. In rare cases, lipemia is present as an expression of increased transport of fat. The increased fat content causes clouding of the serum which, on standing, develops a surface layer which resembles cream. The eyegrounds show **lipemia retinalis.**

Cardiovascular disturbances are frequent. Circulatory collapse and shock may occur (p. 308). In severe acidosis, the pulse is small, soft, and rapid; the blood pressure is low; and the veins are collapsed. The blood pressure is an important prognostic sign. Recent studies suggest that depletion of the adrenal cortex is responsible for failure of the circulation. At autopsy, the adrenal cortex often shows marked deficiency of lipids.

In older patients with preexisting nephrosclerosis or ascending urinary tract infections, the renal damage caused by diabetic coma may be so severe that the diabetic coma may be followed by a true uremia ("renal coma").

In the early stages of coma, signs of abdominal irritation may be so severe as to simulate peritonitis (so called "diabetic pseudo-peritonitis"). The diagnosis of diabetic coma must then be differentiated from other causes of peritonitis, including perfora-

ted ulcer, vascular thrombosis, acute pancreatitis, etc. Occasionally, the urinary amylase is even increased. Patients who have died at this stage fail to show peritonitis at autopsy. When such symptoms and signs occur at the beginning of diabetic coma, therefore, the physician must avoid surgery and instead treat the coma promptly and energetically. Only if adequate therapy does not relieve the coma should operative intervention be considered.

The prognosis of diabetic coma depends on early institution of therapy (p. 306). The longer coma persists, the poorer the prognosis. An important criterion, as already discussed, is the blood pressure: the prognosis is poor if the pressure continues to fall despite adequate therapy. The onset of arrhythmia is an ominous sign. Extreme elevations of the blood acetone (over 70 mg%) and non-protein nitrogen (over 200 mg%) almost always connote a hopeless prognosis.

Principles of Treatment of Diabetes

In the final analysis, all discussions of pathogenesis have as their aim the successful treatment of diabetes. The goal of all forms of therapy is the same: to reestablish a normal metabolism which may once again fulfill its function of providing energy through the metabolism of fats and carbohydrates and thus support life. Modern means of therapy have been so successful in attaining this goal that the diabetic has a long and useful life. The ultimate proof of the success of diabetic therapy will be the elimination of the dreaded late complications: in this regard, our task has only just begun.

Prior to the introduction of insulin, the approach to therapy always consisted of reducing the intake of carbohydrates. The necessary calories were then supplied instead by large amounts of fat (Petrén, Allen). The resulting high-fat low-carbohydrate diets are of purely historical interest today. For reasons which are, in retrospect, difficult to comprehend, this type of diet was championed even after the introduction of insulin, and despite the demonstration by Adlersberg and Porges in 1926 that a low-fat high-protein diet is not harmful (and could be beneficial) to the diabetic. A similar view had already been held by von Noorden. It was only after the experiences of the starvation years from 1943 to 1949 that a diet high in carbohydrates and low in fats was more widely accepted in Europe. Prior to this time, only a few clinicians (Bertram, Katsch, Rabinowitsch, etc.) had used such diets, which actually resemble the diets of healthy individuals. In recent years, most diabetic clinicians have recognized the value of this type of diet (Duncan, Grayzel and Warshall, Steigerwaldt, etc.). However, a few authors are still unwilling to accept the new concept. Thus, Grafe in Germany has continued to defend the high-fat diet, and even today he considers the modern concepts of diabetic diets merely a "passing fashion."

These different views concerning the proper dietary treatment of diabetes are largely due to varying concepts of what constitutes optimal regulation of the state of metabolism. In former times, it was deemed necessary to return the metabolism of the diabetic to normal by appropriate treatment with diet and insulin; that is, to return the blood sugar to normal and to make the urine sugar-free. Grafe, Joslin, Bürger, Sherill, and a number of others still make this demand today. Bürger claims that, if any glycosuria remains, other abnormal products of metabolism, including unknown vasotoxic substances, must be present within the body (p. 272).

Even with the use of insulin, it is seldom possible to attain this goal of normal blood sugar and glucose-free urine, and then only if the diabetic ingests relatively small

amounts of carbohydrates. The concept of "carbohydrate tolerance" should be mentioned here; by this is meant the amount of carbohydrate which the body is still able to utilize without producing glycosuria.

Ideally, the calories of the diabetic should be distributed as they are in the normal diet. The attempt to maintain the blood sugar within normal limits and the urine free of glucose should be made by distributing the food properly under the protection of insulin. This ideal goal, however, is largely utopian, since in practice insulin therapy is always more or less unphysiological. In recent years, the goal has been successfully reached in certain cases of counter-regulation diabetes by the use of the sulfonylurea derivatives (p. 308), which apparently permit the patient's remaining insulin to have a full effect once more.

The high-fat, low-carbohydrate diet always carries with itself the potential danger of acidosis, with consequent impairment of physical and mental capacity. In addition, the forcible return of blood glucose to a normal level by means of insulin is accompanied by the prolonged risk of hypoglycemic shock. For these reasons, we have taught for some time that it is safer to maintain the blood sugar at a slightly raised (but, if at all possible, constant) level and to accept a small degree of glycosuria. The aim of treatment, we feel, is not the lowering of the blood sugar, but the maintenance of carbohydrate oxidation and adequate synthesis of glycogen. The glucose equivalent of insulin increases as the intake of carbohydrates goes up: a high-carbohydrate diet makes sense if only for this reason. The resulting diet is quite similar to the normal diet of the non-diabetic and keeps the diabetic capable of work. Of the carbohydrates ingested, as much as possible should be utilized by the body. It is better for the diabetic if, for example, of 250 grams of carbohydrate ingested, 20 grams are excreted (utilized: 230 grams), than if he were just free of glycosuria on an intake of 100 grams (utilized: 100 grams). We firmly believe that control of the diabetic with production of such a good "balance" or utilization of carbohydrates is to be desired, more so than a normal blood sugar and absence of glycosuria. However, not all workers in the field concur.

The claim that small degrees of hyperglycemia and glycosuria ultimately lead to impaired glucose tolerance and to earlier development of late complications has not been substantiated. On the contrary, John has recently reported that 7 diabetics observed for 14 to 37 years showed no deterioration of glucose tolerance despite prolonged hyperglycemia.

The opponents of the modern therapy of diabetes continue to refer to animal experiments which are advanced to prove the danger of hyperglycemia. However, these experiments, which go back to Dohan and Lukens, must be interpreted with caution. They are stimulating as regards theories of the pathogenesis of diabetes, but they have unfortunately caused confusion with regard to the therapy of human diabetes. The animal experiments are conducted with unphysiological doses of glucose, anterior pituitary, and adrenocortical hormones; if they contribute anything at all towards our understanding of problems of therapy, it is at best that acute burdens on the pancreatic islands can cause exhaustion of this tissue. Properly executed modern therapy attempts to avoid such acute burdens.

The optimal regulation of the diabetic must be carried out in such a manner that the carbohydrate balance remains favorable with, at the same time, avoidance of unnecessary variations of the level of blood sugar, acidosis, and hypoglycemic shock. The fasting blood glucose must not exceed 160 to 180 mg%, and the glycosuria 30 grams in 24 hours. The return of the serum cholesterol to normal values indicates

good diabetic control (White). These goals are best attained by means of a high-carbohydrate, low-fat diet with moderate amounts of protein.

As Macleod has stated, carbohydrates are the fuels of life. All energetic processes are dependent on the oxidation of carbohydrates (p. 193). It is the maintenance of this oxidation, and not the lowering of the blood sugar, which is the ultimate goal of diabetic therapy. The larger the amount of carbohydrate ingested, the better the carbohydrate utilization: the glucose equivalent of insulin rises (p. 296). Diabetics whose high blood sugar levels have been forcibly made "normal" often complain of generalized malaise, headaches, lack of energy, and depression; these complaints disappear when the blood sugar again rises. A high-carbohydrate diet prevents acidosis, and inadequate intake of carbohydrates is one of the commonest causes of coma. Hypoglycemic shock can best be avoided by the use of a high-carbohydrate diet. There is, however, an upper limit above which the carbohydrate intake of the diabetic may not go, even if insulin is given. This level is usually 350 grams (in exceptional cases, 400 grams) of carbohydrates per day.

We emphasize that a "runaway" metabolism must be avoided at all costs. For this reason, we strenuously object to the "free diet." The free diet is always accompanied by marked variations of the level of blood sugar, which severely burden the islet tissue and favor the change of latent to overt acidosis. The free diet probably also favors the development of the late complications (p. 273), and for this reason should also be rejected in juvenile diabetes.

At the same time that the carbohydrate intake is increased, the intake of fats must be sharply curtailed. Fats probably promote the onset of arteriosclerosis (p. 270) and the overt appearance of a latent diabetes (p. 262). During the years of starvation in Germany, acidosis and coma were rare. When the economic reform of 1948 brought about a small rise in the amount of fat in the diet, these complications suddenly appeared again. Acidosis induces resistance to insulin, and fat decreases the glucose equivalent of insulin. The years of starvation were characterized by diets always low in fat and sometimes very high in carbohydrates: during these years, the insulin requirements of diabetic patients were lower than in times of normal nutrition (Heinsen).

When the diet is rich in carbohydrates, no diabetic is sensitive to protein. The daily intake of protein may and should be adjusted to the needs of the body. Protein which is not needed for anabolic processes, such as growth, is partly used for gluconeogenesis (p. 202). In the late vascular complications and in juvenile diabetes, large amounts of protein are necessary to counteract hypoproteinemia and to provide sufficient protein for anabolic processes.

Proper diabetic control also requires the intake of adequate amounts of vitamins. The vitamins of the B complex are building blocks of the coenzymes, which direct the normal sequence of carbohydrate and fatty acid metabolism (Fig. 41, p. 130). An increased intake of carbohydrates might increase the requirements for the B vitamins, and when they are lacking the patient may develop insulin resistance, diabetic neuritis, and ariboflavinosis. Vitamin C is said to take part in the synthesis of the adrenal cortical hormones.

The intake of minerals must also be adequate. Magnesium is necessary for phosphorylation; manganese, for certain steps of the metabolism of proteins. Some metals are incorporated into enzymes (zinc, iron), and zinc is also a component of insulin. The normal absorption of glucose require the intake of adequate amounts of salt; the action of insulin depends on the same substance.

Dietary Therapy in Diabetes

A proper diet is the foundation of every diabetic regimen. Our fundamental principle is that the nutrition of the diabetic should be as close as possible to that of the non-diabetic. The diet of the general population is low in fat, high in carbohydrate, moderate in proteins, and high in vitamins; that of the diabetic should be the same, **modified to a degree by the chronic deficiency of insulin.**

The diet should not contain more than the necessary amount of calories. Both Bouchard's dictum, "Eat as little as possible," and von Noorden's advice, "Moderation in all things," are in accord with this principle. Obesity must be avoided or, if present, must be vigorously attacked. Of especial importance are the following three principles:

(1) Pure sweets are forbidden, for these are quickly absorbed and produce marked variations in the blood sugar.

(2) A diabetic whose weight is normal should take no more than 60 to 80 grams of fat daily. Obese diabetics should have no more fat than absolutely required by culinary technics.

(3) Permitted slowly-absorbed carbohydrates (bread, potatoes, fruits) must be distributed over 5 or 6 meals throughout the day, to guarantee the continuous oxidation of carbohydrates and the synthesis of glycogen.

These precepts must be followed even in patients who are very busy. Some of the carbohydrates must be supplied by fruit, which contains easily utilized fructose and large amounts of vitamins. The amount of carbohydrate to be ingested is determined in each case by the age of the patient, his state of nutrition, and his physical, mental, and emotional problems. We use an average of 250 grams per day, with a range between 150 and 350 grams. For reasons which we have already discussed (p. 288), we reject in principle attempts to nourish diabetics even temporarily without carbohydrates. Grafe, Joslin, and others also no longer treat the patient initially by fasting or an overly rigorous diet.

Diets are becoming more and more simple to use. The mental acrobatics of the older nutritional systems can be discarded. We use a standard diet, which can be readily modified in individual cases. Other special diets are needed only rarely, chiefly in decompensated diabetics with severe glycosuria and acidosis. The doctor himself should tell the diabetic what he should eat, and the rules of the diet must be followed strictly. A scale is no longer necessary, except perhaps for an occasional check. It should be pointed out that the carbohydrate content of individual foods is subject to variations, as through variable moisture of bread, varying grades of ripeness of fruit, etc. Tables of nutritional values can be done away with. The best measure for the correct number of calories is the body weight. The preparation of the food should be simple, and normal food normally prepared should be offered. Books with hundreds of special recipes are unnecessary. Diabetic foods are not, in general, necessary or advantageous. From the very beginning of treatment, the normal life of the patient should be taken into account, and the meals and regimen arranged accordingly.

The Standard Diet

It is desirable to use the standard diet from the very beginning, with insulin as necessary. Our standard diet contains 250 grams of carbohydrates, 70 grams of fat, and 80 grams of proteins, and constitutes 2,000 calories. Table 57 illustrates the basic principle—restriction of fats plus uniform distribution of permitted carbohydrates.

Table 57. A Standard Diet for Diabetics (Bertram)

	Carbohydrate 250 grams	} = 2,000 calories
	Protein 80 grams	
	Fat 70 grams	
Breakfast:	150 grams whole wheat bread, 20 grams butter or margarine, lean cheese, tea or coffee, dash of milk	
Late morning:	medium apple or other fruit	
Lunch:	soup	
	lean meat or fish	
	20 grams fat – salad or vegetables	
	125 grams potatoes	
	125 grams apple or other fruit	
Mid-afternoon:	50 grams whole wheat bread	
	10 grams butter or margarine	
	tea or coffee, dash of milk	
Dinner:	lean meat, fish, or cheese; salad or vegetables	
	125 grams potatoes	
	100 grams whole wheat bread	
	10 grams butter or margarine	
Evening:	125 gram apple or other fruit or 1 glass (8 oz) apple juice	

The feedings between meals should consist of fruits. Such high-carbohydrate low-fat diets can be used even in the presence of complications, and even in the presence of tuberculosis it is wise to avoid giving more fat (p. 285).

Pure sugar, sweets of all kinds (including honey, which is 80% sugar), and pure glucose are interdicted because of their rapid absorption and effect on the blood glucose. Any necessary medications must not be coated with sugar. Saccharine, Sucrets, and similar sweetening agents may be used. Sweetening agents which also have nutritional value are fructose and sorbitol, of which 30 to 50 grams daily may be allowed, depending on how well they are tolerated by the patient. In severe cases, impaired tolerance may follow the use of fructose, but dividing the daily amount of fructose into several small amounts eliminates any difficulty. Other carbohydrate derivatives such as pentoses, caramel sugar, galactose, and dihydroxyacetone are unnecessary.

It is simplest to give the carbohydrates in grams or equivalent units. In certain parts of Germany, bread units (BU) and white bread units (WBU) are sometimes used: 1 WBU = 20 grams white bread = 12 grams of carbohydrate as pure starch; 1 BU = 25 grams of whole wheat bread = 12 grams of carbohydrate as pure starch. Table 58 lists the chief carbohydrate-containing foods according to their carbohydrate content, and thus allows the exchange of one food for another.

The principle sources of carbohydrates in diabetic nutrition are vegetables, fruits, bread, flour, and potatoes. Milk and many desserts also contain carbohydrates. The carbohydrate content of vegetables is not calculated in the diet. In general, legumes are forbidden and artichokes, which are rich in bound fructose, have recently been highly recommended. The vegetables, of course, also contain vitamins and minerals. At least some of the vegetables should therefore be eaten raw or in the form of salads. Canned, dried, and frozen vegetables are all permitted.

At least 50 grams of carbohydrate per day should be in the form of fruit, which contains easily utilized fructose and many vitamins. Fruit must be ripe but not overly ripe. However, dried fruits such as dates and figs, sweet cherries, bananas, grapes, and fully ripened pears are forbidden. Lemons, grapefruit, squash, and rhubarb need not

be calculated. Nuts contain large amounts of fat, which must be considered in preparing a diet. Fruits should be eaten raw whenever possible, and several varieties of fruits in season (not merely one type of fruit) should be included in the diet each day. Small amounts of marmalade are permitted, and small amounts of fruit juices. Table 58 gives the average carbohydrate content of the common fruits.

Whole wheat bread, which contains vitamin B₁ and other vitamins, is recommended. Special breads and pastries need not be used. Diabetics have been known to overstep their diets because of the misconception that special breads may be eaten in unlimited amounts. White bread is perfectly satisfactory. Soya bread has a very low carbohydrate content and would be excellent, but it has a poor taste and has not won general acceptance. Soya meal may be used as a supplement to other breads. Instead of bread, certain flours, cereals, and rice can be used in like amounts - e. g., oatmeal for breakfast. The anti-ketogenic effect of "oatmeal days." (p. 294) is based on the principle of one-sided carbohydrate nutrition; in the framework of the total nutrition oats have no different effect from other carbohydrate cereals.

The potato is a good source of vitamin C, rich in proteins and potassium, and relatively cheap.

Milk and milk products are permitted within the framework of their carbohydrate content (Table 56). Milk may be taken in small amounts in coffee, tea, or cooking without adding it to the calculations. Unsugared condensed milk is permitted, but cream must be calculated because of its content of fat. The carbohydrate content of cheeses need not be calculated, but the fat content must be. Cream cheese is especially recommended.

The problem of alcohol is a difficult one. In small amounts, alcohol is completely oxidized and thus exerts an anti-ketogenic effect. Too much alcohol, however, can not only disturb the metabolic equilibrium but lead to liver damage. Beer, champagne, and liqueurs are interdicted. Recently marketed diabetic beers and diabetic champagnes have, it is true, a low carbohydrate content, but we are against their use in principle. If a diabetic has become used to diabetic beers and champagnes, there is always a danger that he may drink non-diabetic liquors with deleterious results. Today's diabetic diet is so liberal that it is not necessary to expose the patient to potential dangers, and diabetic liquors should be avoided. In former years, certain wines were allowed in small amounts, but their sugar content has been artificially

Table 58. Carbohydrate Exchange Table

25 grams of carbohydrate are present in	
50 grams of whole wheat bread, graham bread, pumpernickel	
40 grams of white bread, rolls	
30 grams of cereals (wheat, rye, oats, barley, rice)	
50 grams of legumes (peas, beans)	
125 grams of potatoes	
250 grams of apples, pears, cherries, peaches	
400 grams of oranges, plums	
500 grams of strawberries, apricots, quince	
600 grams of blueberries, raspberries	
2 glasses (1 pint) of unsweetened fruit juice (apple, raspberry, strawberry)	
2 glasses (1 pint) of milk, skimmed milk, or buttermilk	
No bananas, grapes, beer, liquor, wine	
30 grams = 1 ounce	

increased, and the term "nature-pure wine" no longer guarantees a low carbohydrate content. A few wine companies still produce fully fermented wines whose carbohydrate content is declared on the label, and these may be taken in moderation (1 glass daily). Small amounts of brandies, cognac, and whiskey are permitted. The diabetic brandies on the market differ from the normal brandies only by an increased price.

Vinegar and mustard may be used as spices. Salt restriction is indicated in the diabetic only for the same reasons as in the non-diabetic. Juvenile diabetics must not be kept on low-salt regimes for any period of time.

Coffee and tea are permitted. Diabetic chocolate should be avoided for the same reasons as diabetic beer and champagne. Coca-cola and similar drinks must be forbidden because of their high sugar content.

Mineral waters are often claimed to have a good effect in diabetes (p. 316), but, if there is any such effect, it is certainly exaggerated.

Of the fats which are allowed, we give half as butter or margarine and half as animal fat or oil. It is difficult for the cook of the family to prepare foods with the fat minimum of 40 grams a day because of the "hidden fat" in meat, fish, etc.

The daily intake of protein should not be less than 80 grams, with 50 to 60 grams as animal protein because of its high content of essential amino acids. Fifty grams of meat equal 10 grams of protein; 1 egg or 50 grams of cheese equal 6 grams of protein. The diabetic may have only lean meat, lean fish, and non-fatty cheeses, especially cream cheese, whose carbohydrate content can be ignored. Older patients with diabetes should not eat more than 1 or 2 eggs daily.

Special Diets

In febrile conditions and certain other complicating disorders with anorexia, it is sometimes necessary, in order to avoid the development of acidosis or hypoglycemic shock, to give pure glucose or pure fructose, or foods which are ordinarily prohibited, such as honey and easily digested crackers, such as Zwieback. Such foods are absorbed rapidly, and when they are used the patient should receive no more than half the carbohydrates allowed in the standard diet. These foods should be distributed uniformly through the day. An ounce (30 grams) of honey or Zwieback contains 25 grams of carbohydrate.

Raw vegetables and vegetarian diets can be used temporarily in diabetics for weeks or, at most, months, if desired. If given for longer periods of time, they may promote the appearance of late complications because of the absence of biologically valuable proteins (p. 273). In juvenile diabetics, deficiency of salt may result in resistance to insulin.

Kempner recommends his rice diet, originally used for hypertension and arteriosclerosis, for diabetes, too. He has claimed that the rice diet not only exerts a favorable influence as regards the vascular complications, but also lowers the blood sugar and reduces the requirement for insulin.

Anti-ketogenic diets are indispensable in the treatment of diabetic acidosis. These are based on von Noorden's observation that pure carbohydrates, after an initial rise in blood glucose, correct acidosis and the imbalance in metabolism. "Oat days" (von Noorden), or better, "oat and fruit days" are used for their anti-ketogenic effect. Falta's cereal days are seldom used today. The oat diet must be low in fats, and consists of 150 to 200 grams of oats daily in five or six servings (soup, pudding, pastry). Lemons, cucumbers, tomatoes, green salad, and red wine may be used for flavoring.

The "wet and fruit days" include raw or cooked fruits *ad lib*, and are often a pleasant change for the patient. The large content of fructose is the basis for their anti-diabetic, anti-ketogenic effect. Patients with gastric or intestinal disturbances may also benefit by "wet days," and those with diarrhea by Morro's diet of grated apples.

The "free diet" was introduced in 1933 by Stolte for the treatment of juvenile diabetes, and is still widely used by a number of clinicians (Weisse 1951; Larson, Lichtenstein and Thomsen 1952; Engleson 1954). We, in common with others, reject the concept both for the diabetes of the adult and especially for juvenile diabetes, because it permits uncontrollable deterioration of metabolism and favors the development of capillary damage (p. 273). The term "free diet" is somewhat confusing. John recommends the term "liberal diet" and Fanconi speaks of a "normal diet." Stolte's free diet permits all sweets. Stolte's measure for the nutrition of juvenile diabetics is their appetite. He administers insulin 3 to 4 times a day. Bossert also uses the free diet, his only restriction being that the children not eat "too many candies and sweets."

The Use of Insulin in Diabetes

It was 32 years after the discovery of pancreatic diabetes by Mering and Minkowski that an effective insulin was first produced by Banting and Best by extraction of animal pancreases. The time interval was long because at first it was not possible to separate the hormone from the enzymes of the pancreas. Zuelzer had prepared an insulin long before Banting and Best — in the year 1905 — but it could not be used therapeutically because of the toxic effects of insulin shock which were not understood at that time. With increasing understanding of diabetes, and with improved use of diets, insulin therapy has undergone many changes. New types of insulin were developed and have permitted the use of large amounts of carbohydrate. For the patient, insulin has been a miracle drug, and no diabetic need die of diabetes itself as long as insulin is available.

The mechanism of action of insulin has been discussed (p. 209). Dosage is given in biological units. The standard preparation is crystalline insulin, which was first prepared in 1925 by Abel: 1 mg — 24 international units. Each insulin must be tested on diabetic patients before it may be marketed. Control is vested in insulin committees of various countries.

Insulin cannot be used in any pre-ordained, stereotyped manner. It may be given in a single daily injection only in cases in which the patient's metabolic state does not show wide variations. The diabetic must learn to inject himself through personal instruction by his physician. Appropriate insulin syringes calibrated to 40 or 80 units per cubic centimeter are necessary, or more simple syringes divided into 10 equal parts. The syringe can be kept sterile in 70-75% alcohol, since it is used for the same patient and the same drug.

Insulin must be administered parenterally. All attempts to give insulin by mouth have met with failure (p. 315).

The literature suggests that 50 to 60% of all diabetics require insulin, and Joslin claims that the figure is nearer to 85%. In our own clinic, we use insulin for 85% of diabetics, but in some of these insulin is used only temporarily. Absolute indications for the use of insulin are diabetic coma and the pre-comatous state, complications of diabetes which have caused metabolic deterioration, juvenile diabetes, and diabetes in which diet alone fails to control the diabetes and, particularly, to prevent the devel-

opment of ketone bodies (p. 289). Insulin can also be used temporarily in older diabetics to improve their metabolic status, and in new cases occurring during the climacteric. In such cases, it may be possible to correct the functional disturbance of the relationship between the A-and B-cells and thus to return the diabetes to the stage of latency (p. 302).

There are no contraindications to the use of insulin. Special care, however, must be employed in the presence of cardiovascular disorders, especially hypertension, coronary sclerosis, and retinopathy. In such patients, hypoglycemic shock may be especially harmful because of the danger of hemorrhage.

The types of insulin available include regular insulin, depot insulin, and mixed insulin (depot plus regular insulin). Stated differently, there are 3 insulins which act rapidly (regular, crystalline, semi-lente), 3 which are intermediate in action (globin, NPH, lente), and 2 which are long-acting (protamine zinc, ultra-lente). The long-acting insulins which have been recently developed have as their aim the use of a single injection daily, but this aim cannot be attained in all cases. Each insulin has its own indications.

In uncomplicated cases, the dose should if possible not exceed 80 units in 24 hours. For most patients, 20 to 60 units are enough. In complications, the amount of insulin necessary may temporarily increase. The diabetic on insulin must never omit insulin because of lack of appetite or for other reasons. Improper interruption of insulin treatment is today the most common cause of the development of coma (p. 286). With experience, it is possible to estimate the amount of insulin required fairly accurately, and to start immediately with the calculated dose (p. 297). Change in the type of insulin used often causes temporary deterioration of the metabolic state.

Insulin therapy is controlled by determining the fasting blood sugar and the excretion of glucose in the urine in a 24 hour period. Only patients who are difficult to regulate require repeated determination of daily blood sugar, or blood sugar determinations during the course of the day.

Regular Insulin

Regular insulin is a clear, acid, aqueous solution of insulin, and is given by subcutaneous injection. It is absorbed rapidly and acts rapidly, its effect starting after 1 hour and being complete after 3 to 4 hours. When regular insulin alone is used in the control of diabetes, frequent small injections are therefore necessary, and a carbohydrate meal must be taken 30 to 45 minutes after each injection. Each dose should not exceed 24 units.

Regular insulin is indicated where a rapid effect is important: in coma, in all types of complications, in operations, during childbirth, etc. In new patients, we usually start with regular insulin and later change over to longer acting insulins. The amount of insulin necessary is estimated by means of the "glucose-equivalent of insulin"—i. e., the amount of glucose brought to utilization by one unit of insulin. The glucose equivalent does not have a constant value, but is higher, for example, in insulin-deficiency diabetes and lower in counter-regulation diabetes. The higher the blood sugar, the higher the glucose equivalent. The glucose equivalent rises with increased intake of carbohydrates and falls on a high-fat diet. It is especially high when the insulin is given at night. The average glucose equivalent is given by the formula, 1 unit of insulin permits utilization of 2 grams of glucose. Thus, if a diabetic excretes 70 grams of glucose daily, we try to cause oxidation of 60 of these 70 grams, using

20 units of regular insulin in 24 hours, divided into 3 doses of 10 units each given before each main meal.

When glycosuria is severe, a higher glucose equivalent is used in order to avoid insulin overdosage: 3 grams per unit of insulin when the glucose excretion exceeds 120 grams, and 4 grams when the excretion exceeds 200 grams. Thus, if a patient excretes 250 grams of glucose, we compute that he needs 60 units of insulin on the basis of a glucose equivalent of 4 grams per unit. In such a case, the patient receives 20 units in the morning, 16 units at lunch, 16 units at dinner, and 8 units at 2 a. m.

The time of injection of insulin is of importance. Often the doses given throughout the day are insufficient to produce a satisfactory equilibrium in diabetics whose insulin requirement is high. This is especially likely to be true if the normal rhythm of glycogen formation is disturbed (Möllerström). In the healthy individual, the glycogen content of the liver is high in the morning (low blood sugar) and lowest towards noon (high blood sugar). During the evening and during the night, the glycogen again increases (blood sugar decreases). This rhythm is disturbed in many diabetics so that the blood sugar has already begun to rise in the evening hours and reaches very high values the following morning. This "morning peak" of the blood sugar increase can be eliminated, according to Staub, by a small dose of insulin given at night. If the daily rhythm starts with a low blood sugar, the glycosuria also decreases. The glucose equivalent of the night dose of insulin is especially high. It is often possible, by redistributing the time of the insulin injection (i. e., by using an injection at night), to cause improvement of the equilibrium of the body (Fig. 66).

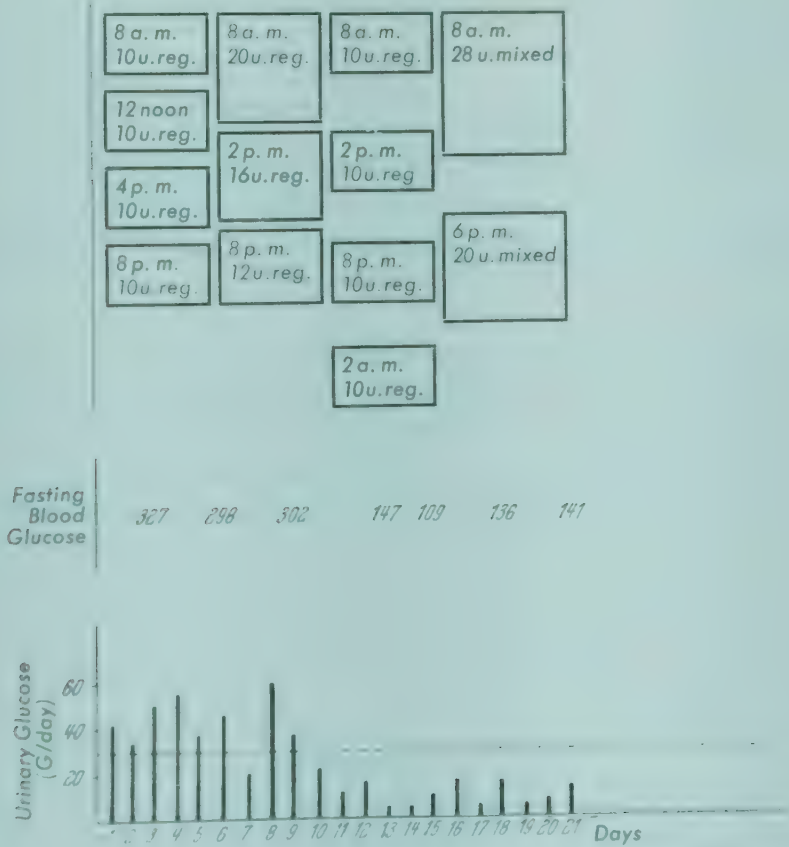


Fig. 66. Management of a diabetic with regular insulin. There is marked reduction of the glycosuria with the use of insulin at night.

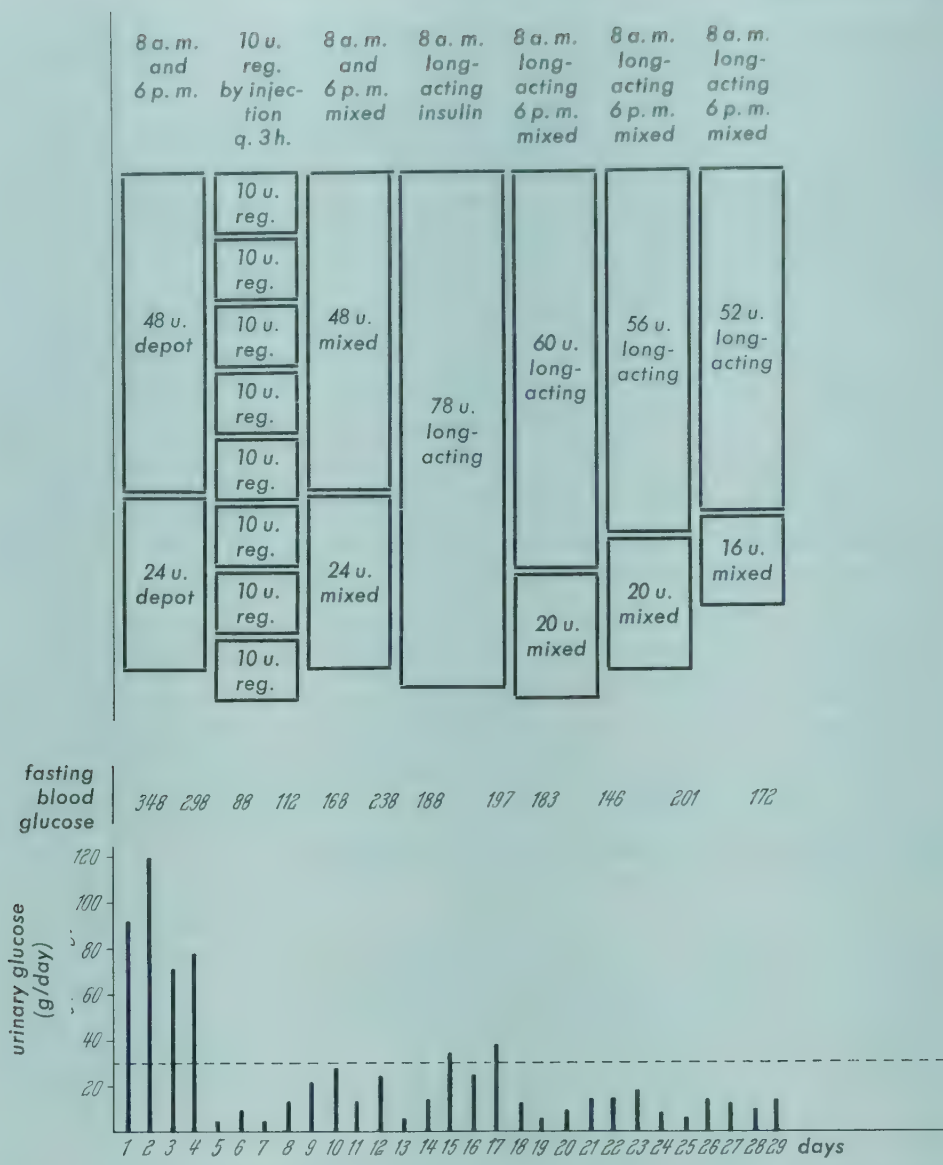


Fig. 67. Management of a diabetic with repeated injections of insulin ("Stosstherapie") followed by unification of doses.

These nightly injections have not become entirely superfluous with the development of long-acting insulins. We use them initially in the regulation of moderately severe and severe cases, in the treatment of coma, and in the treatment of acidosis. Repeated, frequent injections of regular insulin are also used temporarily in acidosis and in patients whose metabolism cannot be equilibrated in any other way. Regular insulin is given every 3 to 4 hours throughout the day and the night, each dose consisting of 8 to 10 units (Figs. 67, 69). Such therapy is continued until the metabolic situation was under control, usually 2 to 6 days, and then the frequent small doses are combined into a smaller number of larger doses. Still later, a change is made from regular insulin to a long-acting insulin.

Depot Insulins

The concept of depot insulins arose in 1925. At that time, we ourselves noticed an increase in the action and duration of effect when insulin was mixed with proteins. This effect was absent when insulin and protein were injected separately. From this we concluded that the change in effect must be due to prolongation of the absorption

of the insulin by the protein. We also felt that the type of material used to prolong absorption was of importance. Unfortunately, we did not study these problems further. In 1936, Hagedorn succeeded in producing practical long acting or depot insulins.

Depot insulins have a protracted action which is due to the modification of regular insulin in such a way that its absorption by the body is prolonged. The different effects of a given dose of regular and depot insulin are illustrated in Fig. 68.

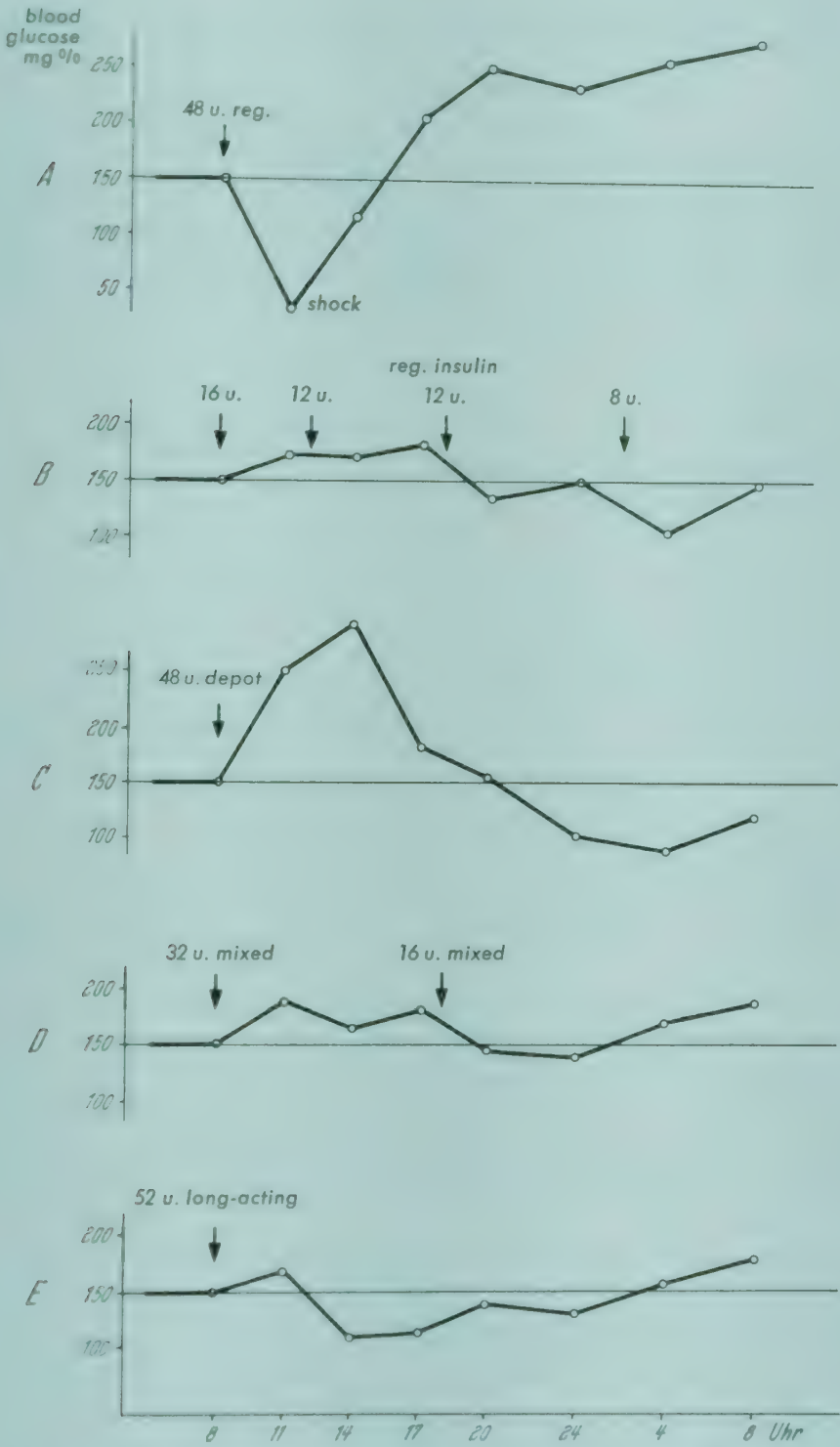


Fig. 68. The action of the same dose of various types of insulin.

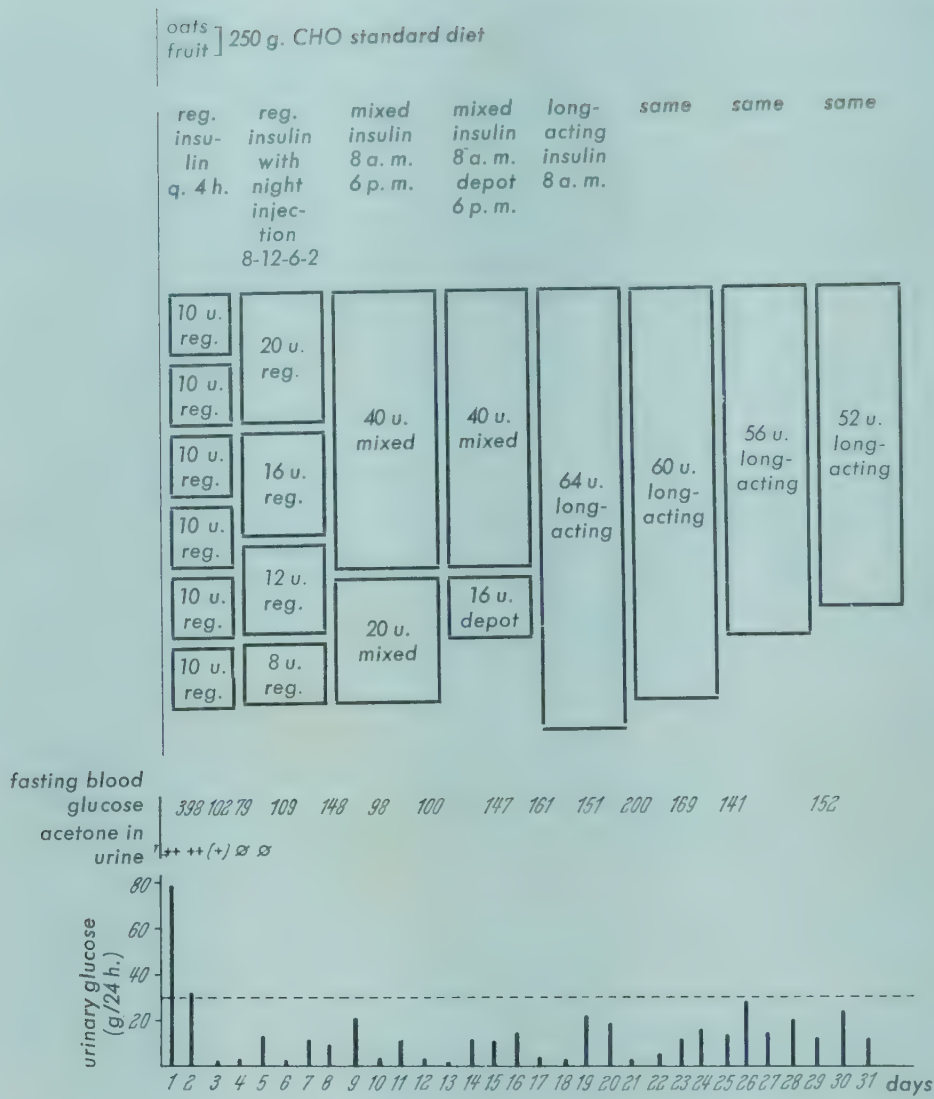


Fig. 69. Management of a patient with diabetes by means of regular insulin followed by change to mixed insulin and then to depot insulin.

Intermediate-acting insulins include globin, NPH, and lente insulins. Long-acting insulins are protamine-zinc insulin and the crystalline insulin zinc suspension known as ultra-lente. Protamine-zinc insulin has a prolonged effect with a maximal decrease of blood sugar after 12 to 14 hours. Surfen-insulin (a bis-quinoline protein-free product) causes maximal decrease of blood sugar after 6 to 8 hours, and is very useful provided that it is recognized that 2 injections a day are needed.

No more than 40 or 50 units of the depot insulins should be given in a single dose. The distribution of carbohydrates must be carefully watched when the depot insulins are used, special care being taken that the largest amounts are given in the afternoon and evening hours.

Depot insulins are useful in long-term therapy, but are not indicated in conditions which demand rapid and prompt action. If diabetics regulated with depot insulins develop complications which threaten their metabolic state, one must switch promptly to regular insulin in repeated doses (Fig. 69). The advantage of depot insulins lies in the small number of injections required. In very mild cases, a single injection may be enough. In more severe cases, two are generally necessary: a larger one in the morning, and a smaller one in the evening. Since depot insulins have a more uniform action

than regular insulin, the patient can get along with about two thirds of the regular insulin dosage. When regulation is good, the blood glucose shows especially good uniformity.

Some authors (Heinsen et al.) use depot insulins from the very beginning of treatment. Our own experience is that it is better to begin with regular insulin in order to avoid overdosage, and then switch to depot insulin. When changing from regular to depot insulin, the patient may sometimes show severe glycosuria. Such a finding should not, however, lead immediately to an increased dose, but one should rather wait several days to allow the body to accustom itself to the insulin effect. Depot insulin, in contrast to regular insulin, can be given independently of mealtimes.

Combination and Mixed Insulins

The term "combination insulin" refers to preparations in which regular and depot forms of insulin are present in unchanged form next to one another. One such combination insulin consists of equal parts of crystalline insulin and a form of depot insulin known as "isoinsulin," and is known as "Di-Insulin."

The term "mixed insulin" refers to a mixture of the 2 component insulins to form a new insulin whose duration of action lies between that of regular insulin and that of the depot insulin. Certain types of mixed insulin are commercially available (e. g., "Komb-Insulin Hoechst" in Germany), but others may be made up by the patient. Thus, for example, we like to use a "day insulin" which consists of 2 parts of regular insulin plus 1 part of depot insulin. (The corresponding "night insulin" consists of 1 part of regular insulin and 2 parts of depot insulin, and is the commercial Komb-Insulin mentioned above.) When the patient makes mixed insulin, a little trick is necessary. First, regular insulin is aspirated into the syringe. Then, to equalize the pressure, a sterile needle is inserted into the rubber cap of the depot insulin ampoule and stays there while the depot insulin is withdrawn. In this way, regular insulin is prevented from being sucked into the ampoule of depot insulin. Examples of the use of mixed insulins are shown in Figures 67, 68 and 69.

The tendency toward hypoglycemic shock is less with mixed insulins than with depot insulins, for unknown reasons, so that larger doses of mixed insulins may be employed. The dose should not exceed 60 units at any one injection, however, under any circumstances.

Crystalline Suspensions of Insulin

Mixed insulins offer an excellent method of regulation of diabetes, but 2 injections may be required in some cases. Newer insulins have constantly been developed with the ultimate goal of one injection per day, the hope being that some insulin be found whose absorption during the day might be more rapid and during the night more prolonged. Such insulins can be used only after the patient has been optimally regulated by means of regular, mixed, and depot insulins. The diet must be modified to conform with the insulin effect: often, it is necessary to give a high-carbohydrate meal in the afternoon and to reduce the carbohydrate content of the morning meals by a corresponding amount. If infections or other complications occur, one must return to regular or mixed insulin temporarily.

Several types of crystalline suspensions are available. *NPH 50 insulin* (Hagedorn) derives its name from N = neutral reaction, P = protamine zinc, H = Hagedorn's

improvement, 50 = the amount of protamine (0.50 mg. per 100 units). This insulin is only partly saturated with protamine. Its effect may last for 28 hours, and doses up to 120 units have been used (Marble and collaborators) with good results from a single daily injection even in juvenile diabetics.

"Long-insulin" was prepared by Dörzbach and Lindner (Hoechst Company of Germany) and clinically tested by us. It consists of insulin with varying amounts of added surfen. Different crystals are present in the same aqueous solution, giving a graduated depot action. This material is effective in a single daily injection in 60 to 70% of diabetics, including those who are moderately severe and severe. Shock is rare, but we never use more than 80 units in a single injection.

The Lente Insulins. The lente family of insulins was introduced by Hallas-Möller, Jershild, Petersen and Schlichtkrull. These insulins are based on the observation that one can modify absorption at will solely by changing the size of the crystals and by adding zinc. Interfering ions, including phosphate, citrate, and others, must be absent. Semi-lente is rapidly acting, though not as rapid as regular insulin; lente is intermediate acting, similarly to NPH and "long-insulin" described above; and ultra-lente is long-acting.

Special Indications for Insulin

Temporary Treatment with insulin in older diabetics can improve metabolic equilibrium and lead to a general increase in the sense of well-being which may continue after insulin is discontinued. Latent acidosis, which can be present even without ketonuria, is the usual cause of metabolic deterioration in such patients, and is treated by small doses of regular insulin at the beginning, switching to depot or mixed insulins later. The result is a "pancreas vacation," which may be continued even for weeks, and then insulin discontinued slowly.

In diabetes with onset in the climacteric, prompt treatment with insulin may return the disorder to latency within a matter of weeks, especially in obese men aged 50 to 60 years, less often in menopausal women. In our opinion, the diabetes in these cases is at first due to a purely functional disturbance of the relationship between the A- and B-cells with a relative increase of the A-cells; elimination of this disturbance prevents permanent damage of the B-cells and thus prevents the development of a permanent form of diabetes. Such patients sometimes show resistance to insulin which is due to hyperfunction of the anterior pituitary. Such resistance can be overcome by the simultaneous administration of sex hormones (100–200 mg. testosterone in a period of 2 weeks).

Surgery in diabetics carries little more risk than in non-diabetic patients, because of improvement in management, the availability of various forms of insulin, and close cooperation between internist and surgeon. Acidosis may be precipitated by restriction of the diet, the operative procedure, or anesthesia, and it is the responsibility of the internist to prevent the onset of acidosis. Since ether anesthesia causes severe hyperglycemia, it is preferable to use intravenous, local, or gas anesthesia. Curare is not contraindicated. If food cannot be taken by the patient, he should receive multiple intravenous and subcutaneous infusions of glucose under the protection of insulin (e. g., 20 to 40 cc of 20–50% glucose intravenously; 1 to 2 liters of isotonic glucose by hypodermoclysis). The operative and postoperative periods require the use of regular insulin as indicated.

Reduction of the Dose of Insulin

It is a mistake to withhold insulin from regulated diabetics for any reason, or to reduce the dose of insulin too quickly. Even in complete starvation, the patient should continue to receive one-half to two-thirds of the regular dose of insulin. The commonest cause of coma is improper reduction of insulin dosage (p. 287). If exogenous insulin is discontinued too rapidly, the production of remaining endogenous insulin which is present in every diabetic ceases, and the entire metabolism collapses.

Resistance to Insulin

Insulin resistance is present if the diabetic metabolism does not react or reacts only slightly to parenterally injected insulin with a lowering of the blood sugar appropriate to the dosage. Several causes may be distinguished.

Absolute insulin-resistance is extremely rare. We have seen this only once, in a case of bronzed diabetes (p. 281).

Partial insulin-resistance is fairly common, and is quite characteristic of counter-regulation diabetes (p. 258). In many cases, sex hormones may eliminate this resistance to insulin (p. 314). In others, X-irradiation of the pituitary may be of value, but only in diabetics with signs of pituitary disturbances. In extreme cases of "long-term diabetes," Luft found a disappearance of insulin-resistance after hypophysectomy. Insulin-resistance also occurs in the presence of tumors of the adrenal cortex, in hyperthyroidism, and in cases of extra-insular (renal) glycosuria.

Severe but temporary resistance to insulin can occur in acidosis. The classic example is diabetic coma, in which huge amounts of insulin may be required initially (p. 307). It is assumed that the insulin is destroyed either by the acidosis itself or through the action of the adrenal cortex. The inadequate effect of insulin after the ingestion of large amounts of fat is partly due to a latent acidosis without acetonuria (p. 290).

Concomitant diseases may also be responsible for resistance to insulin: carcinoma, cirrhosis of the liver, disorders of the pancreas, and particularly all types of infection. The mechanism of action is not known. The tryptic enzymes of the increased leukocytes were formerly held responsible, but today adrenal cortical dysfunction (Selye) is considered the basic cause of the resistance to insulin. To overcome the resistance in complications, large amounts of insulin must be given temporarily. Insulin-resistance can also be precipitated by vitamin deficiency, especially by deficiency of the B-vitamins, and can then be corrected by the administration of riboflavin and nicotinamide. In addition, Bornstein, Trewhella, and others have described an anti-insulin factor in the serum of patients who show resistance to insulin.

Hypersensitivity to minimal doses of insulin is the opposite of insulin-resistance. Such hypersensitivity is often found in juvenile diabetics, in rare cases of cirrhosis of the liver, and often in patients with advanced glomerulosclerosis (p. 278). Marked reactions to insulin are also seen in the rare combination of diabetes and Addison's disease, of which 46 cases have been described in the literature (Stanton et al.). As Addison's disease develops, the requirement for insulin decreases.

Dangers of Insulin Therapy

In uncomplicated diabetes, no tissue damage has been reported with the correct use of insulin. Insulin is, however, a powerful drug and should be used with care in diabetics with cardiovascular damage (p. 296).

When insulin is first started, one occasionally sees transient insulin edemas, especially in diabetics with gastric anacidity. As a rule, hyperchloremia is present, never a diminished blood chloride. Falta, however, believes that the sodium ion is the responsible agent, for in salt-free diets edema occurs if sodium bicarbonate is given, but not potassium bicarbonate. The patients complain of temporary disturbances of vision because of presbyopic anomalies of refraction (p. 276). Insulin must, however, be continued, preferably with a low-salt, low-fluid diet. If necessary, diuretics can be used.

Allergic reactions occur occasionally. In the last few years these have been more frequent and more severe than previously. They generally occur on the 5th to 10th day after the beginning of therapy, chiefly as local reactions, but sometimes as severe generalized urticaria together with swelling of the mucosae. Gastrointestinal symptoms (diarrhea) may be prominent. These allergic reactions are often due to the agents which are used to prolong the action (i. e., delay the absorption) of insulin, and sometimes to the added antiseptic agents. When the reaction is purely local, the alcohol used to sterilize the syringe may be responsible: the syringe should be rinsed with boiled water prior to use. Rarely, the insulin molecule itself is the cause of the allergic reaction. In such cases, the physician must desensitize the patient, using, for example, specially prepared insulins which have undergone several recrystallizations.

Lipodystrophy due to insulin is a harmless but annoying local reaction. Insulin lipodystrophies may be degenerative, especially in women, or lipomatous, especially in men. They are generally seen at the site of the injections, but may occasionally occur in parts of the body which have never come in contact with the insulin. They are thought to be neurogenic trophic disturbances due to stimulation of the autonomic nervous system. Children, and women between the ages of 30 and 50, are especially affected; men much more rarely. The incidence in our experience is 15 to 20% of diabetics. Both atrophy and lipomas may arise at the same time. Insulin injected into such lipomas is poorly absorbed, and imbalance of the metabolism may follow. Frequent change of the injection site is recommended to avoid insulin lipodystrophies. The changes often disappear if the insulin is injected into the deepest part of the lipodystrophic tissue (Günther), or sometimes if vitamin B is administered.

Hypoglycemia. Severe insulin shock must be avoided at all times. Occasional mild episodes of hypoglycemia cannot always be avoided during the phase of regulation, but these also demand close observation of the patient. If they become frequent, deterioration of metabolism will follow. Permanent brain damage may result from "therapy-resistant insulin shock" (Keymling), but this is fortunately rare. Such shock sometimes causes vascular spasm or stasis in the vessels of the central nervous system; at other times, it produces changes in the nerve cells, especially those with the extremely sensitive Nissl substance. The histopathologic changes include small hemorrhages and diffuse changes in the nerve cells, especially in the cortex, corpus striatum, thalamus, midbrain, cerebellum, and medulla oblongata. The clinical picture thus shows marked variability, and may include myoclonia, hemiplegia, convulsion, Korsakov syndrome, or dementia.

The clinical picture of shock is different when it follows regular as opposed to depot insulin. The picture of spontaneous hypoglycemia and hyperinsulinism is discussed elsewhere (p. 319).

Hypoglycemia after Regular Insulin. The hypoglycemic syndrome which follows regular insulin is not directly related to the height of the blood sugar. It depends rather on the speed of fall of the blood sugar and the compensatory "counter-regulation" measures produced, especially the release of epinephrine from the adrenal

glands (p. 266). Shock after regular insulin can thus occur even though the blood glucose is normal or, rarely, even increased.

The first symptoms are muscular weakness and excessive appetite. Soon, general agitation, sweating, and palpitation supervene as a consequence of epinephrine release, and collapse may follow. Convulsions are common in animals, but rare in human beings. Mobilization of glycogen depots may cause severe acidosis. In severe cases of overdosage with insulin, and especially in labile diabetics, the dominant picture may be cerebral, because of deficiency of glucose in the brain. Such symptoms may vary from slight irritability to severe delirium, and from slight fatigue to coma. Hypoglycemia after regular insulin may also manifest itself as sudden syncope without prodromal symptoms or signs. In general, hypoglycemic reactions can be precipitated or aggravated by unusual degrees of muscular effort (p. 319).

Hypoglycemia after Depot Insulins. The hypoglycemic syndrome after depot insulin is due to deficiency of glucose at the tissue level, and generally manifests itself only when the blood glucose has sunk to extremely low values. Compensatory mechanisms either do not occur or do not occur to the same extent as after regular insulin, because the rate of fall of the blood glucose is much slower.

Clinically, the patient shows listlessness, depression, and migraine-like headaches. Secondary palpitation, agitation, and sweating—epinephrine signs—are lacking. In severe cases, the same cerebral symptoms as after regular insulin may be present. In some cases, "silent shock" may occur during the night (Katsch): the patient does not know of this, but complains the next morning of pressure in the head and giddiness. In such cases, the morning fasting blood glucose may be quite high (compensatorily), and the nocturnal glycosuria considerable.

The differential diagnosis between hypoglycemic shock with unconsciousness and diabetic coma may be difficult, the determination of blood glucose being the surest distinction. In hypoglycemia, the breathing is virtually normal, and there is no acetone odor to the breath. In diabetic coma, the breathing is deep and continuous, and the acetone odor of the breath is obvious. In hypoglycemic shock, the pulse is slower and often irregular; in coma, it is small and rapid. In shock, the skin and the oral mucosa are moist; in diabetic coma, they are dry. In hypoglycemic shock, the eyeballs are firm; in diabetic coma, the eyeballs are soft and sunken. Other findings in some cases of hypoglycemic unconsciousness include a staring look when the eyes are raised, diplopia, and a bilateral Babinski sign.

The treatment of hypoglycemic states consists of the administration of large amounts of glucose. If the patient can drink, he should be given fruit juices which have been sweetened with glucose or fructose. In other cases, concentrated glucose must be given intravenously (e. g., 20 c.c. of a 50% solution). In many cases, a prompt response occurs and confirms the diagnosis, but it must be emphasized that occasionally a single dose of glucose is not enough, and the patient must be under continued observation. In severe cases, an additional injection of 0.5 mg epinephrine subcutaneously may be helpful. If the diagnosis between diabetic coma and hypoglycemic unconsciousness is in doubt, insulin must not be used under any circumstances, but a therapeutic dose of glucose can be given for test purposes. Such a dose is not harmful in diabetic coma.

Every diabetic who receives insulin must understand hypoglycemic shock and must carry fruit or sugar with him at all times. He should also bear an identification card which includes his name, his doctor's name and telephone number, and his dosage of insulin, and his diet.

Habituation to insulin "insulinism" does not exist. If, during treatment, insulin dosage must be increased, some underlying cause must be sought (e. g., low grade infection).

Therapy of Diabetic Coma

The common denominator of all cases of diabetic coma is a critical deficit of insulin. The mainstay of therapy, therefore, is the use of insulin. The therapeutic problem in diabetic coma, however, is not merely to combat the hyperglycemia and glycosuria, but to correct the acidosis and the disturbances of water and electrolyte balance which produce the dehydration. In addition to insulin, a number of ancillary measures are needed for this purpose (Table 59).

In treating a patient with coma, it is imperative to follow the metabolic changes continuously, day and night, by clinical and repeated laboratory determinations, including blood glucose, urinary sugar, ketonemia, alkali reserve, NPN, and blood electrolytes.

The dosage of insulin in coma differs in different clinics. It is necessary to distinguish between the initial doses necessary to overcome resistance to insulin (p. 303) and later doses. In former times, the amounts of insulin given initially were often inadequate. Joslin reports that the mortality from coma decreased from 18% to 3% when the dosage of insulin was increased from 83 units to 250 units in the first 3 hours. On the other hand, cases are reported which required "heroic doses" (Falta) to overcome coma, from 2,000 to as much as 53,000 units, partly by vein. Grafe reports that 2,000 to 3,000 units may be necessary in some cases. In our opinion, such large doses indicate that complications must have been present. If complications are not present, we

Table 59. Therapy of Diabetic Coma (Bertram)

1. Insulin

High doses initially, part by vein, part subcutaneously; from 40 to 100 units depending on severity of the case, repeated as necessary. We do not use "heroic doses."

Also, small doses at the same time; i. e., 10 to 12 units of regular insulin subcutaneously every 30 minutes to every 2 hours, as indicated.

2. Glucose

Given immediately together with insulin, partly as 50% glucose intravenously, partly as 4.8% glucose by hypodermoclysis. We add 10 units hyaluronidase to each subcutaneous infusion.

3. Salts

Sodium chloride as physiological saline or Ringer's solution; also, in part, as 10% hypertonic saline intravenously.

Alkali as bicarbonate or lactate may be given, but is usually unnecessary.

Potassium only in the presence of severe dehydration, when EKG shows evidence of hypokalemia.

4. Early treatment of any complications which may have produced the coma.

5. Adrenal cortical hormones

Percorten or similar preparations, given 1 hour after the start of treatment, repeated after 4 or 5 hours.

6. Food

Oral feeding of a high-carbohydrate, low-fat diet as soon as possible.

7. Circulatory stimulants

8. General measures

Warmth. Enemas. Gastric lavage in certain cases. Nursing care.

consider such large doses not only unnecessary but even dangerous, and are in this regard in agreement with Katsch, Hegglin, Heinsen, Constam, and others. We do not see how they can otherwise be effective in regulating the metabolic state, especially when given intravenously. In our own clinic, we give 200 to 300 units during the first 24 hours in mild cases, and generally not over 500 to 600 units in severe cases. Our highest dosage of insulin was given to a patient with an infection and amounted to 1,210 units. Katsch uses even smaller amounts: 100 units in the first 10 to 11 hours, 160 units in 24 hours.

Insulin must be started promptly, even before admission to a hospital, by the patient's physician. From 20 to 60 units of regular insulin, depending on the severity of the case, should be given subcutaneously. In the hospital, 40 to 100 units of regular insulin are then given under close supervision of the blood sugar level, partly by vein and partly subcutaneously. The portion given intravenously should be kept as low as possible and should be given together with glucose. Intravenous insulin is especially indicated in the presence of cardiovascular complications or edema. In severe cases, the initial dose must often be repeated one or more times.

Following the first large dose of insulin, we then administer repeated, small doses of 10 to 12 units of regular insulin at intervals of one-half to three hours ("Stosstherapie"). As coma subsides, the interval between these injections is gradually increased. Still later, we switch from regular to depot or mixed insulins (p. 298). This regime of repeated small doses of insulin is more physiological than single large doses, spares insulin, prevents compensatory endocrine effects, and avoids the danger of hypoglycemic shock.

The old dispute regarding the use of glucose at the start of therapy of diabetic coma has not been resolved, and clinicians have had different opinions at different times. On the face of it, it seems logical that since, in coma, there is an excess of glucose in the body, additional glucose should not be used. However, it has been repeatedly shown that patients who receive glucose respond more rapidly and lose their coma more quickly than patients treated without glucose. Falta previously opposed glucose, but now recommends it from the very beginning of treatment (1944). Joslin and Katsch use glucose only after the blood glucose has been returned to normal.

The arguments pro and con have been reviewed by Bertram (1953). If given early, the manner of administration of glucose is of prime importance. In all cases, overburdening of the already burdened circulation by large infusions, whether intravenous or subcutaneous, must be avoided. We have used 20 to 40 c.c. of a 20 to 40% solution of glucose with the first intravenous dose of insulin, plus 500 to 1,000 c.c. of a mixture of Ringer's and isotonic (4.8%) glucose solution subcutaneously. Hyaluronidase may be added to promote absorption. We repeat the subcutaneous infusions as indicated, giving a total of 100 to 120 grams of glucose in the first 8 hours by all routes. As soon as possible, we switch to oral carbohydrates. We have never seen side effects with this method.

Fluids and sodium chloride must be given promptly to counteract the deficiency of chloride which is usually present and which is in part responsible for the insulin-resistance of the comatose patient. Again, it must be emphasized that large infusions may overburden the circulation. We ourselves use a subcutaneous infusion of 250 to 500 c.c. of a solution containing physiological sodium chloride plus an equal volume of isotonic glucose solution. In exceptional cases, intravenous infusions must be given over a prolonged period of time; in such cases, the speed of infusion must be controlled so that the circulation is not overloaded. Sodium chloride can also be given by mouth when the patient has been aroused from coma.

In severe cases, blood transfusion may be life-saving.

Some authors recommend the use of alkali to help counteract the acidosis, giving 20 grams of sodium bicarbonate in 500 c.c. of water. In our own experience, we have found this unnecessary.

The addition of potassium may prevent circulatory collapse in some cases. Potassium loss is seldom evident at the start but may become manifest as therapy progresses and dehydration occurs (after 10 to 12 hours). Clinically there are gasping, sudden muscular weakness or even flaccid paralysis, and typical changes in the electrocardiogram (lowering of the T wave, prolonged QT). Potassium chlorate can be given by mouth, 0.5 grams every 2 hours to a dose of 4 to 6 grams per day; or intravenously as a 2% solution, totalling 1.5 grams per day. In our experience, potassium has rarely been needed.

Adrenal cortical hormones in the form of the total extract can promote retention of salt and water, prevent loss of potassium, and maintain the blood pressure. Thus 10 mg of Percorten can be given subcutaneously 1 to 2 hours after the start of therapy and again several hours later. Some authors (Markees and Meyer; Boulin) have had good results from cocarboxylase, which is phosphorylated thiamin. Neither we nor Joslin, Lawrence, Krainick, or others have confirmed these findings.

Symptomatic treatment of the circulatory system is widely used in Europe, seldom in America. Joslin considers such therapy superfluous. We, and many other clinicians, continue to employ circulatory stimulants and strophanth in severe cases to support the blood pressure and prevent circulatory collapse. We do not, however, use intravenous analeptics to stimulate consciousness. Special measures consist of warmth (the patient is often hypothermic), catheterization of the bladder, and the use of enemas as indicated. Severe meteorism may sometimes be seen, due to marked dilatation of the stomach which can be detected clinically and by x-ray. In these cases, gastric lavage with warm water through a nasal tube is indicated, but with care because of the danger of collapse.

Oral Therapy of Diabetes Mellitus

General Remarks

Two groups of chemical compounds are available today for the oral therapy of diabetes mellitus: the sulfonylureas and the guanidine compounds. The sulfonylureas were first used in the form of the sulfonamide BZ 55 which was synthesized by Haack, and later also in the form of the non-sulfonamide D 860. Unfortunately, these substances were introduced into medicine under various names (see Table), and we propose to use only the designations BZ 55 and D 860 in the present survey to avoid any confusion.

Chlorpropamide has recently been marketed in the United States under the name of Diabinese. Metahexamide is being tried out at present on a large scale. Both these preparations can be given in smaller doses than is possible in the case of BZ 55 and D 860. The lower dosage is explained by the delayed excretion and the resultant prolonged retention period within the body (half-life). The therapeutically effective blood levels of metahexamide are considerably lower than those of the other sulfonylureas, and the half-life of this compound lies between that of BZ 55 and that of D 860. The lower dosage, in other words, is the result of a true potentiation of effect.

The guanidine compounds are closely related to Synthadin which was introduced into therapy in 1927 by Frank but which had to be withdrawn because of severe renal and hepatic toxicity. The guanidine compound currently in use bears the generic name "Phenformin," and is known in the United States as DBI.

Chemistry

The following table shows that the two groups of compounds are not related chemically in any way:

Oral Antidiabetics

Sulfonylureas

BZ 55	Sulfonamide (acetylated in the body) 1-butyl-3-p-sulfanilylurea Nadisan, Invenol, Carbutamide, Glucidoral, Alentin, Orabetic
D 860	Non-sulfonamide (oxidized in the body) 1-butyl-3-p-tolylsulfonylurea Rastinon, Artosin, Tolbutamide, Dolipol, Orinase
WP 40	3-amino-4-methylbenzenesulfonylcyclohexylurea "metahexamide"
P 607	1-propyl-3-p-chlorobenzenesulfonylurea Chlorpropamide, Diabinese

Diguanidine compounds

DBI	β -phenethylformamidinyliminourea-HCl
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Mode of Action

The mode of action of the two groups is entirely different. Although it has not been possible as yet to explain the mode of action in any detail, we are nonetheless aware of certain important differences which are essential for establishing the indications for the various compounds.

The efficacy of the sulfonylureas is dependent on the presence of an adequate number of undamaged β -cells in the islet tissue. The efficiency of these cells is increased by the sulfonylureas. A second point of action of the drugs has been found to be the enzyme systems of the liver; the sugar secretion from the liver is reduced and the amount of liver glycogen is increased by these drugs.

It is assumed that the guanidine compounds exert their effect independently of the pancreas; that is to say, they are active even when the β -cell system has been destroyed. The hypoglycemic effect of these compounds is brought about by a reduction of glycogenesis and an increased sugar uptake by the muscles accompanied by reduced consumption of oxygen. The content of liver glycogen decreases and large quantities of lactic acid are formed.

Clinical Indications

The indications for these groups of oral antidiabetic preparations can be established from these findings.

The primary use of sulfonylurea therapy is in counter-regulation diabetes in elderly people of the sthenic-adipose habitus. It has been shown that in this form of diabetes the β -cells usually remain intact for a considerable period of time. This form of therapy has now been generally accepted by the medical profession.

For all practically purposes, treatment with guanidine compounds is worthwhile only in cases of insulin-deficiency diabetes, which is seen chiefly in juvenile diabetics of the asthenic types. It is doubtful if the guanidine drugs will ever play as important a part in the treatment of this form of diabetes as the sulfonylureas do in counter-regulation diabetes.

Historical Development

Certain parallels can be observed if we compare the historical development of oral therapy in the two groups of compounds.

It was in 1941 that Savagnone first described the hypoglycemic effect of the sulfonamides, which at that time were used as bacteriostatic agents. Although Savagnone considered the possibility of using them in the treatment of diabetes, he did not pursue this thought. In the same way Loubatières, who in numerous publications attempted to solve the mystery of the hypoglycemic sulfonamide IPTD (p-aminobenzenesulfonamide-isopropylthiodiazole), did not carry out his first therapeutic trials until he had learned of our initial findings on BZ 55. We are in honor bound to state that it was Professor Franke and his assistant Professor Fuchs who carried out the first clinical trials, but after the untimely death of Professor Franke we continued the experiments and established the indications for the use of sulfonylurea compounds in diabetes mellitus. The first investigations with BZ 55 in animal experiments were made by Achelis and Hardebeck. The first clinical trials with D 860 were made by Stoetter.

The guanidines were first used as antipyretics. When Frank introduced them for use in the therapy of diabetes in 1926, their hypoglycemic action was already known. The drug he used, Synthalin (decamethylene-diguanidine) proved to be highly toxic. As early as 1929, Hesse and Taubmann investigated the nature of several of the guanidine compounds and gave a detailed description of their action which is very similar to that of DBI. At the time they did not try out these drugs on human subjects "because of the toxic component found."

In 1957, Ungar, Freedmann and Shapiro first published the results of pharmacological studies of the guanidine DBI in the United States. In the same year clinical reports were published by Pomeranze, Williams and Krall. These reports described the successful use of this substance and some of its derivatives in human subjects. We have been able to confirm their results concerning the effects of DBI in diabetes.

Clinical Data

1. Sulfonylureas

General

The use of sulfonylureas in the treatment of juvenile diabetics and markedly asthenic elderly patients should be ruled out at the very beginning. In cases that are severely decompensated, and in the presence of acidosis, a short course of insulin is first advisable (4 to 8 days) and this period should be followed by changeover to oral treatment. It should be realized, however, that any oral therapy is dependent on strict adherence to a proper diet. Oral and intravenous tests have been described to establish whether a diabetic is likely to respond to sulfonylurea therapy, but the value of these tests is open to dispute and we ourselves believe that they are unnecessary.

Dosage

BZ 55 and D 860 are catabolized within the body in different ways. BZ 55 is acetylated and has a half-life in the serum of 48 hours. D 860 is oxidized and has a half-life of only 8 hours. These figures refer to the period of time that elapses before half of the substance disappears from the organism. They are a good relative indicator of the duration of effect and they involve certain variations in dosage.

In the early days of oral diabetic therapy, it frequently happened that overdoses of sulfonylureas were given. This error must be avoided at all costs. Not more than 2.5 grams (5 tablets) of BZ 55 should be administered on the first day of treatment and this dosage should be reduced in the course of the next 4 to 5 days until 1.0 gram is reached. When continuous treatment is used, an attempt should be made to stabilize the patient on 0.5 or even 0.25 grams daily. If it is found that 1 gram is insufficient to achieve stabilization, changeover to insulin must be made. The daily maintenance dose of BZ 55 may be given in one dose, preferably in the morning after breakfast.

The initial doses of D 860 are higher, owing to the fact that it takes longer for the effects to become apparent. Three grams (3 times 2 tablets) are given daily in the first 4 to 6 days. In continuous therapy the maximum of 1.5 to 2.0 grams per day should never be exceeded. Since D 860 is excreted at a rapid rate, the doses should be evenly distributed throughout the day.

It should be realized that doses of BZ 55 and D 860 in excess of those mentioned above are not only useless but in certain circumstances they may even be harmful to the patient since they provide favorable conditions for the onset of side-effects.

Results

We have treated some 2,000 diabetics with sulfonylureas. The chances of success depend on the accurate assessment of the indications.

Of the sthenic elderly patients (above the age of 35), 80% were treated successfully. The remaining 20% who failed to respond to treatment consisted almost exclusively of diabetics who had been treated for a prolonged period with fairly large doses of insulin. Such insulin treatment results in atrophy and degeneration of the β -cells in many patients. Since it is impossible to predict in advance the extent to which the β -cells have already been damaged, treatment with sulfonylureas should be attempted even after a prolonged period of insulin treatment.

More than 95% of the new cases of diabetes in this group of patients responded satisfactorily to the oral preparations. We have now reached the conclusion that diabetes in elderly people should not, in the majority of cases, be treated with insulin, with the exception, of course, of emergency indications.

It is worthy of note that even in the group of asthenic elderly diabetics (over 35 years of age) as many as 40% responded satisfactorily to treatment.

It frequently happens that combined treatment with insulin and tablets yields unexpectedly good results; i. e., it is possible to dispense with a large proportion of insulin injections and sometimes it is even possible, in carefully controlled steps, to discontinue insulin altogether.

In rare cases, the sulfonylureas were also found to be temporarily effective in cases of juvenile diabetics, the figure being 10% of all cases in our studies. These, however, were all new cases of diabetes in juveniles, and in these patients it is certain that the

β -cells are still intact at the beginning. Later on, however, they degenerate, either rapidly or gradually (metamorphosis). In general, however, treatment with sulfonylureas should not even be attempted in juvenile diabetes.

Late Failures

In most cases of diabetes, the sulfonylureas produce satisfactory control over a prolonged period of time, but in some cases their efficiency gradually falls, necessitating a temporary or even permanent return to insulin therapy. Figures given in the literature vary between 5 and 10%.

Non-observance of diet or complications of diabetes, such as infections, myocardial infarctions, and cardiac insufficiency, are the causes of apparent late failures. In these cases, it is almost always possible for oral therapy to be resumed after stabilization has been achieved by dietary measures and, where necessary, insulin, and after the elimination of the accompanying diseases. It is a fundamental error to assume that a dietary lapse may be rectified by taking more tablets. All diabetics who wish to enjoy the benefits of oral medication must be prepared to pay for this by strict maintenance of the diet. The diet is as important in this regard as it is in insulin therapy.

True late failures of unknown origin are extremely rare. In any case, it is certain that they are not due to exhaustion of the β -cell system. True late failures occur chiefly in cases in which the oral treatment was of uncertain prognostic effect to begin with (asthenic diabetics, patients with a long history of insulin therapy). It is typical of many of these cases that the action of the tablets was largely wasted in any case; that is to say, that it was neither possible to achieve aglycosuria nor to reduce the fasting blood sugar levels to near normal values. It would be more accurate to speak of "primary failures" in these cases. Despite oral medication, stabilization deteriorates because the change from counter-regulation diabetes to insulin-deficiency diabetes could not be prevented. In these cases, guanidine therapy may be attempted. If this also fails, further therapy should be with insulin.

In view of the above, we may say that there is no reason to assume that the efficacy of the sulfonylureas is in any way limited, provided that the original indication is accurately assessed.

Side Effects

Fortunately, the sulfonylureas have few side-effects, and those that they have are relatively harmless. Side effects are more frequent with BZ 55 than with D 860. They are almost invariably harmless, and there is no need to deprive the profession of BZ 55 since it is more reliable than D 860 and the necessary dosage is smaller. In our case material there has not been a single fatality nor has any permanent damage due to side effects occurred. The picture is more serious even as regards insulin, owing to the ever-present danger of insulin shock. It is very rare for shock to occur with the sulfonylureas, and in any case such shock is always harmless. The incidence of gastrointestinal disorders is practically nil. The most frequent side effects are allergic skin reactions (3.2% of the cases after BZ 55 and 0.6% after D 860), and it is not always necessary to discontinue treatment because of them. In some cases, allergies have occurred after the use of both drugs. There have been some cases of leukopenia. In our 2,000 cases, we have seen only one case of agranulocytosis, which responded to the usual therapy. We have not seen thrombocytopenia. In spite of a temporary effect

on the intestinal flora following the use of BZ 55, there have been no cases of intestinal disorders. Reports have appeared in the United States of frank hepatic damage after the use of BZ 55, necessitating discontinuation of therapy. We have seen no such cases, nor have they been observed in any other country where BZ 55 has been used. This fact leads us to the assumption that there must be other factors which cause this "complication" in the United States (high fat diet, abuse of medications with resultant allergy). Mention should be made of certain harmless side effects such as temporary disturbances of vision at the beginning of therapy (which also occur after insulin treatment), and intolerance to alcohol.

Four years of experience and investigations in many thousands of cases have recently been reported at the 3rd International Diabetes Conference in Düsseldorf, and we are now in a position to state that it seems highly unlikely that any posttherapy organic injury may occur in the future. It is not yet possible to make any conclusive statements about the effects of oral therapy in connection with delayed action or late complications.

2. Guanidines

We have treated 100 diabetics with DBI. The guanidines are effective both in counter-regulation diabetes and in insulin-deficiency diabetes. However, they are of no practical value in the counter-regulation diabetes group because the sulfonylureas, which can be used for these patients, are more reliable and safer. It is interesting to note, however, that a few patients showed satisfactory stabilization with guanidine compounds after late failure had occurred with the sulfonylureas.

In the insulin-deficiency diabetes group, the efficacy of the guanidines is limited according to the insulin requirements of the patient.

DBI, used alone, is effective in a small number of new cases of juvenile diabetes with a low initial insulin requirement.

The most important use of DBI is in combined insulin-DBI treatment of patients with fairly high insulin requirements (30 units or more), which cannot be covered by DBI alone. In these cases, the additional DBI therapy results in a reduction of insulin requirement so that it becomes possible, for example, to give one injection daily instead of two. More important is the fact that it is often possible to eliminate the unpleasant fluctuations in blood glucose level which occur with juvenile diabetes.

Thus, the indications for the use of the guanidines are rather limited. Furthermore, it has not yet been possible to establish how long these substances remain effective; i. e., it is impossible to predict the possible percentage of late failures. Our own experience suggests that this percentage will by no means be negligible. Finally, unpleasant side effects are likely to occur in 50 to 60% of the cases treated. However, even the prolonged use of the drug, in excess of one year, has never resulted in any true toxic organic changes. On the other hand, these side effects often demand cessation of therapy. The side effects include queasiness, nausea, diarrhea, and later on (after 4 to 10 weeks) weight loss, physical debility, and generalized malaise. The extent of these side effects depends on the dosage, and they can be controlled if oral treatment is cautiously instituted and not more than 2 tablets (100 mg) of DBI are given daily.

Summary

The vast amount of literature on the subject of oral diabetic drugs is a reliable indicator of the importance of this type of therapy. Oral diabetic therapy is a great step forward in the treatment of this disease and has now been generally accepted

by the medical profession. This is particularly true of the sulfonylureas. Insulin can now be replaced by BZ 55 or D 860 in 35 to 40% of all cases of diabetes. The value of the guanidines has not yet been finally established. The question of their further use will depend on the results of further clinical trials. And, perhaps it will be possible in the future to develop more effective and less toxic substances in this group of compounds.

Surgical Treatment of Diabetes Mellitus

Attempts to increase carbohydrate tolerance by partial extirpation of the pancreas have been without effect. Following total pancreatectomy, the insulin requirement of the diabetic is reduced (p. 281). However, this operation is indicated only under special circumstances, notably carcinoma of the pancreas. Denervation of the adrenal glands with the goal of inhibiting pancreatic antagonists has been unsuccessful. Luft has recently performed hypophysectomy in 10 cases of especially severe diabetes, hoping to influence the progressive late changes. Six of the patients survived from 4 months to 3 years after the operation. In these, the tendency towards acidosis and the insulin requirement fell appreciably. However, the improvement observed does not justify this serious operation at this time.

General Measures in Diabetics

Vitamins. Vitamins are effective only if a corresponding deficiency exists. After the vitamin reserves have been satisfied, they have no further effect on carbohydrate metabolism. Vitamins should be given primarily in their natural form, as part of the diet, but may also be added in therapeutic form.

Hormones. Certain hormones may be of value. This is true primarily for the sex hormones, which can overcome certain types of insulin-resistance (Petrides, Constan). They are sometimes used in the treatment of the late capillary complications of diabetes. The drug p-hydroxy-propionophenone (0.5 grams daily) is said to inhibit the release of certain glandotropic hormones of the anterior pituitary gland, but we have seen results only rarely.

The hormones of the adrenal cortex must be used with great caution. As is well known, ACTH and cortisone themselves cause glycosuria and may precipitate diabetes. A few reports, however, suggest that cortisone may sometimes improve diabetes.

Thyroid, often incorrectly used in obesity, should be avoided in diabetics.

Sedatives. The usual sedative medications may be used in diabetes as in the non-diabetic patient.

Psychology. Psychiatric measures, formal and informal, are of great importance for the diabetic. It is up to the patient's physician to explain the disorder to him, to allay his fears, and to help him over emotional hurdles. The physician must explain and repeat that the controlled diabetic can lead a normal life, but that, at the same time, early and continued control are mandatory to help ensure a long and uncomplicated life (Table 60).

Physical Effort. The diabetic should be encouraged to use his muscles. Muscular effort increases the catabolism of carbohydrates and helps improve the metabolic situation. The amount of insulin, and the caloric content of the diet, depend on the amount of work which the patient does. Work which is tiring, and especially athletic

Table 60. Rules of Life for the Diabetic. From Bertram (1953)

1. Do not announce that you are a diabetic.
2. Stick rigidly to your diet. Use care when you take insulin. Exercise regularly as directed.
3. Avoid obesity.
4. Live a normal, healthy life. "Early to bed, early to rise, plenty of fresh air and sunshine." When tired, go to bed for 15 to 30 minutes. Relax regularly and do not overexercise. Take a normal vacation. Avoid alcohol and smoking.
5. After having had diabetes for 10 years, see an ophthalmologist at least once a year.
6. Take care of your teeth. Use a soft brush twice daily, massage your gums regularly, and see your dentist every 3 months. If necessary, have your teeth replaced in time.
7. Take care of your skin, using mild soap and water, and only the mildest of salves as indicated.
8. Take care of your feet, washing daily with lukewarm water and drying thoroughly, especially between the toes. If the skin is dry, use lanolin salve; if the feet sweat, use powder. Cut your nails very carefully and only after a foot bath. Make sure that your socks are smooth and your shoes soft and fit properly. Do not use hot water bottles or electric heaters. Exercise and massage your feet. If your feet are injured, inflamed, infected, or burned, see your doctor immediately.
9. Have regular bowel habits. Eat fruit, whole wheat bread, and other foods with roughage. If necessary, use a mild laxative. In the presence of diarrhea, use grated apples and unsweetened tea, and see your doctor.
10. Protect yourself from colds and chills. An x-ray of the chest should be made as part of the yearly examination.

activities, must be curtailed or forbidden. Milder exercise, however, is permitted: walking, slow bicycling, horseback riding, etc. Each diabetic must, of course, be individualized in this respect.

The diabetic should be encouraged to lead a normal life and to have a relaxing vacation at least once each year. Modern concepts of diet permit the vacation to be taken almost anywhere, as the patient chooses.

Spas and Resorts. In certain parts of the world (especially, perhaps, Germany), there are resorts which specialize in the treatment of diabetes. In general, success is due chiefly to the psychic changes which accompany the stay at the resort. The patient becomes relaxed in attractive surroundings; he eats well and at specified times; he walks leisurely in the fresh air. Unfortunately, modern therapeutic methods have not found their way to all such resorts, and in the absence of specific therapy the patient may well relapse on his return to his former way of life. Hence, it is wrong to try to reduce the amount of insulin during the time that a diabetic is at a resort, but better to increase his intake of carbohydrates temporarily. Even the healthy person eats more during his vacation, and to reduce the diabetic's insulin during a vacation is merely to subject him to re-regulation on his return home.

Worthless "Antidiabetics"

With the exception of the sulfonylurea drugs, there is no medication which can substitute for parenteral insulin. All attempts to use artificial devices to promote the oral absorption of insulin have been unsuccessful. Insulin plus hyaluronidase is useless (Klotzbücher 1952), as is "pancreasmellin new." The same is true of various tissue extracts. The fresh cell therapy of Niehans is of no use in diabetes (Rietschel). Insulin-

like substances of plants (Collip's "glycokinins") and the various "diabetic teas" have been without value. Preparations of sulfur as well as tiny doses of nickel, cobalt, zinc, magnesium, copper, and other metals, have no effects on metabolism. (However, zinc and magnesium when mixed with insulin increase its effect. See p. 300. Porphyrin derivatives and quinine preparations are without effect. Frank's guanidine derivatives, such as synthalin, were introduced 25 years ago; they seem to induce selective destruction of the A-cells (Kühnau), but must be rejected clinically because of their side effects in increasing the renal threshold, creating resistance to insulin, and causing damage to the gastrointestinal tract, the liver, and the kidneys.

Diabetes Mellitus in Childhood

In principle, there are no differences between diabetes in the adult and in the child. Juvenile diabetes is always due to deficiency of insulin, and usually appears during the years of growth. Before or during the appearance of diabetes, many of these children show increased growth and hypoglycemia. Diabetes is very rare in infants, although it has been reported in a 4½ month old child.

If juvenile diabetes is severe and improperly managed, the syndrome known as Mauriac's syndrome may develop. This consists of reduced growth with marked hepatomegaly, the liver showing large amounts of both fat and glycogen. Mauriac's syndrome may be intermediate between diabetes mellitus and glycogen storage disease. Thus, Hahnhart showed that both disorders have a similar inheritance. Mauriac's syndrome is often found in the presence of other manifestations of anterior pituitary disturbances suggestive of Cushing's disease. There is no doubt that deficiency of insulin and incorrect diet (especially inadequate protein intake) are important in the development of this syndrome. The abnormalities can be corrected by proper therapy of the diabetes, but some authors recommend endocrine therapy with thyroid, anterior pituitary, and sex hormones.

The treatment of juvenile diabetes is governed by the same principles as diabetes in the adult (p. 288). The diet should be rich in carbohydrates and low in fats. The protein requirements of the child are higher than in the adult: 1.5 to 2 grams per kilogram of body weight. A diet which lacks or is poor in protein may favor the development of the late complications (p. 273). As already discussed, we are against Stolte's "free diet," and we believe that pediatricians who still use it are not aware of the subsequent late complications, which occur after the child has reached adulthood.

In regulating juvenile diabetics, it is our practice to permit, at the beginning, a liberal diet with the exception of sweets and excessive amounts of fat. The diet varies, of course, according to age, size, appetite, and general habitus, but the resulting diet is recorded in detail. At this point, modifications may be made, so that the carbohydrates are evenly distributed throughout the day, and fats are restricted to a total of 80 grams per day. Proteins may be eaten ad lib. We thus agree with Fanconi's "normal diet," except that we eliminate his "lightly-sweetened compotes."

Every juvenile diabetic must be started on insulin immediately. In most cases, the dose of insulin must be increased over the course of time, since the disease reaches its particular level of severity only in time. Some cases are best treated with regular insulin, others with depot insulins, and many with mixed insulins. Long-acting forms of insulin are not useful in juvenile diabetics, as already discussed.

Insulin shock must always be avoided, but this is not always easy in labile children. In contrast to adults, hypoglycemic reactions occasionally manifest themselves as tonic and clonic convulsions. Any damage to the brain following hypoglycemic shock may be detectable on the electroencephalogram (Krainick). A residual glycosuria, which must not, however, exceed 30 grams per day, is acceptable as a sort of "buffer" against shock.

Physical exercise is of considerable importance, and requires cooperation among patient, school authorities, and parents. Psychologic and social factors are even more important in children than in adults. Summer camps for diabetics are now a well-recognized social and therapeutic device both in Europe and in the United States.

The prognosis of juvenile diabetes, formerly completely hopeless, has improved considerably in recent years. According to Joslin, the average duration of life after the beginning of juvenile diabetes is 21 years. Sexual maturity occurs normally in most cases. The incidence of infectious diseases, including tuberculosis, has decreased. The prognosis is worsened by the late complications, which may occur earlier than in the adult and are usually very severe. Thus, White studied 380 juvenile diabetics and found capillary damage in 93% (75% retinopathy, 55% renal insufficiency, 7% coronary sclerosis, and 2.5% intracranial lesions). The hope, of course, is that improvement in management may prevent or modify these late complications.

Prognosis and Prophylaxis of Diabetes Mellitus

It is an established fact that the incidence of diabetes throughout the world has increased and continues to increase. To a degree, the increase parallels the degree of industrialization of a country. The highest incidence is in the United States, where Joslin gives an incidence of 1% and suggests that an additional 0.5% of the population has unrecognized diabetes. Wilkerson, as a result of systematic examination of urine, estimates that for each 100 known cases of diabetes, there are an additional 75 to 100 unknown cases. Himsworth calculated that the incidence of diabetes in England would amount to 1% in 20 years. There are no definite figures for Germany, but Knorre, Schliak, and coworkers estimate an incidence of 600,000 to 680,000 cases in an overall population of 66 million (1%).

There has also been an increase in the mortality of diabetes, amounting to an average increase of 27% in the last 15 years (Joslin). The explanation lies in the increased life span of the patient with diabetes, which has risen from 46.7 years in 1914 to 64.7 years today. The average duration of the diabetic state has correspondingly risen from 4.9 to 14.1 years (Joslin). In other words, the diabetic today lives long enough to develop late complications and die of these. The causes of death have also changed: before the discovery of insulin, 50 to 60% of diabetics died in coma. Today, only 10 to 15% die in coma, while 70 to 80% die of late vascular complications. The mortality in younger diabetics has decreased markedly since the discovery of insulin: from 38% in 1938 to a current figure of approximately 6% (Joslin).

There is no "cure" for diabetes in general. Cases of overt diabetes associated with obesity ("counter regulation diabetes") may sometimes be returned to latency after weight reduction, but it is always possible that some such patients never had diabetes at all but merely showed the carbohydrate intolerance which is characteristic of the obese individual.

The course and prognosis of diabetes are dependent, to a large extent, on early recognition and treatment. With proper treatment, the metabolic imbalance stands a good chance of correction. With improper or haphazard management, with no attention to proper diet, and with complications, the need for insulin increases. The prognosis of diabetic coma is good if prompt treatment is given. The prognosis of the late complications, however, remains poor.

A word regarding prophylaxis may be in order. Fajans and Conn did glucose tolerance tests in patients of diabetic and non-diabetic families, and found abnormal results in 19% of the diabetic group and 2% of the non-diabetic group. They then repeated the test following a single oral dose of 100 to 125 mg of cortisone: the corresponding figures were 24% and 3%. Such tests suggest a means of discovering latent cases of diabetes, but we ourselves are not convinced that they are without danger, despite the small amount of steroids used, since they may conceivably lead to earlier appearance of diabetes (p. 314). The guiding light of diabetic prophylaxis was well expressed by Naunyn, "Moderation in all things." The diet should be a normal, balanced diet liberal in dairy foods and vegetables. Excessive sweets should be avoided. Obesity must be prevented. Regular mental and physical relaxation are important, and physical work is desirable. Sedentary occupations are more disadvantageous than those with physical effort. The urine should be checked during routine examinations, during pregnancy, and in infections and other disorders; if glycosuria is present, further tests should be made of blood sugar and, as indicated, glucose tolerance.

The diabetic should receive detailed instruction regarding the nature and treatment of his disease. Joslin has advocated this since 1915, and medical and lay organizations – such as the American Diabetes Association – encourage education of the lay public. Joslin notes the value of education in his 300 diabetic physicians, in whom not a single death due to coma was noted (Joslin 1953; Bertram 1955). Diabetic counseling and welfare centers are in existence, especially in larger cities, but the mainstay of the average diabetic must be his personal physician, aided by necessary laboratory and hospital facilities. Hospitalization of a diabetic should be for as short a time as necessary, since the strict control of the hospital is often difficult to carry over to the home.

Diabetic welfare centers may offer counseling with regard to occupation and marriage. Exact rules cannot be made, since exact predictions of heredity cannot be made. Severe diabetics, we feel, should be advised not to marry, since not only the tendency to diabetes but its severity too can be inherited. When the family trait is present only in one parent, the appearance of diabetes in the children is less common than if both parents have diabetes. If both potential partners have diabetes, marriage should be discouraged. In diabetics who already have late complications, marriage should be strenuously opposed.

The diabetic should avoid occupations which might cause deterioration of the metabolic situation, such as those in which the working day varies (traveling salesmen, actors, etc.) and those in which dietary temptation may be great (cooks, bakers, restaurateurs). Diabetics who use insulin must avoid occupations in which shock may endanger either the patient (construction workers, firemen, furnace workers) or others (chauffeurs, bus drivers, locomotive engineers, pilots, etc.).

Diabetics in most countries have founded organizations with the help of their physicians, such as the International Diabetes Federation. These organizations hold meetings and publish useful lay literature. An international diabetic congress is held at three-year intervals and is attended by lay and medical personnel.

Spontaneous Hypoglycemia and Hyperinsulinism

Spontaneous hypoglycemia is the opposite of diabetes mellitus. There is always relative or absolute hyperfunction of the insulin-producing portions of the pancreas. There may be primary overproduction of insulin, or secondary hyperinsulinism as a result of failure of compensatory (counter-regulatory) mechanisms. Various pathogenesis may occur. The term "hyperinsulinism" should be limited to cases of actual overproduction of insulin as a result of increased activity of the islet cells. In all other cases, the preferred term is "spontaneous hypoglycemia."

Spontaneous Hypoglycemia

Physiologic Hypoglycemias. Hypoglycemia may occur physiologically under a number of different circumstances. In the first 3 days of life, the blood glucose level varies between 15 and 120 mg% because of pancreatic hyperfunction and inadequate epinephrine counter-regulation (Meythaler). The values become normal only after the first week of life, but clinical signs of hypoglycemia are rare. In relation to the size of the body, the pancreas of the fetus is 4 times as large as that of the adult and contains 5 times as much insulin. These differences equalize themselves by the 4th year of life, but the blood glucose remains relatively low up to the 10th year of life. The tendency to hypoglycemia in children is thus greater than it is in adults (Meythaler and Fischer).

Hypoglycemia may also occur following exhausting physical effort (e. g., marathon runners) and as a result of excessive secretion of insulin in a person who first eats nothing for several hours and then eats a large amount of sugar. The functional hypoglycemias which occur in "vagotonic" individuals often have only vague, nonspecific complaints (weakness, headache, giddiness) and hence often go unrecognized. Spontaneous hypoglycemia may also occur during lactation.

During the post-war years of starvation, large segments of the population showed marked decreases of the fasting blood glucose. Thus, Wiele, working in the Ruhr Valley of Germany, reported that within a single year the average fell from 95 mg% to 65 mg%. These changes have been explained partly by glycogen deficiency in the liver and partly by central disturbances in the diencephalic-anterior pituitary system (Bertram 1948). Allen had already seen death due to hypoglycemia in some of his diabetics on starvation regimes. In chronically undernourished diabetics, minimal doses of insulin were found to cause fatal hypoglycemia (Wendt and Arnold, Hornbostel, Höpker). The severity of these hypoglycemias was partly the result of deficiency of glycogen in the liver.

Endocrine Hypoglycemias. Spontaneous hypoglycemias occur in the course of endocrine disorders. The importance of the pituitary was first emphasized by Houssay, whose hypophysectomized dogs were extremely sensitive to insulin. In humans, severe reductions of blood glucose are seen in Simmond's disease, adiposogenital dystrophy, and pituitary infantilism, and less often in Addison's disease and myxedema. Typical hypoglycemic episodes have been reported in occasional cases of adrenal cortical tumors, with profuse sweating and blood sugar values of 24 to 30 mg%. Hypoglycemia has also been reported, in connection with muscular effort, in patients with hyperthyroidism or with hyperventilation tetany. Such hypoglycemia may also be seen in asthenic individuals as a premonitory symptom of subsequent juvenile diabetes.

Hepatic Hypoglycemia. In 1925, Fischer described hypoglycemia associated with liver damage in dogs with Eck's fistula. Severe hypoglycemia has often been reported in association with various hepatic disorders—cirrhosis, chronic hepatitis, acute hepatitis. These are related to abnormalities of gluconeogenesis and gluconeolysis. Spontaneous hypoglycemia in glycogen storage disease is discussed below (p. 326).

Other *Hypoglycemia's* have been noted in patients with gastric disorders and pancreatic diseases. In the former, they are perhaps partly related to difficulties of absorption and partly to mucous irritation (as in penetrating duodenal ulcer). Pancreatitis and pancreatic stones can be associated with marked hypoglycemia, perhaps because of hypersecretion by the islet tissue as a result of a reflex effect on the vagus. These hypoglycemia's subside as the pancreatic disease is cured. The chronic irritation may, we feel, in some cases produce an adenoma, and it may be that islet-cell adenoma is often, not a primary disorder, but a secondary reaction which follows chronic inflammation plus hormonal imbalance (Bertram 1931).

Hypoglycemia with sudden evacuation of the bowels following gastric resection occurs in the so-called "dumping syndrome." In these cases, a high-carbohydrate meal first causes marked hyperglycemia which is followed by severe hypoglycemia. This phenomenon has recently been explained by the absence of the A-cells following gastrectomy; these cells are present in large numbers, not only in the pancreas itself, but also in the stomach and duodenum (p. 268).

Neurogenic Hypoglycemia's are due to disturbances of regulation by the central nervous system, and have been described in epilepsy, encephalitis, brain injury, subarachnoid hemorrhage, and various other intracranial lesions, especially those associated with coma. Hypoglycemia of this sort is common in progressive muscular dystrophy.

Toxic Hypoglycemia's. Hypoglycemia's occur as the result of poisoning with carbon tetrachloride, guanidine, phosphorus, hydrazine derivatives, etc. These are the result either of liver damage or of disturbed central nervous system regulation. Guanidine derivatives have an additional effect, damage of the A cells of the pancreas.

Familial Hypoglycemia has been reported by Anderson.

Hyperinsulinism

Katsch used the term "pernicious insulinism" for hypoglycemia due to primary hyperfunction of the islands of Langerhans. The clinical picture is characterized by progressive episodes which cannot always be properly controlled. Katsch gathered, from the literature, not quite 100 cases up to the year 1948, but a review article by Vosschulte and Becker in 1953 summarized 315 cases. Campbell reported that islet-cell tumors are found in 1 of every 900 autopsies, so that many must be clinically asymptomatic. On the other hand, death from spontaneous hypoglycemia may occur with no abnormal findings at autopsy.

Islet-cell adenomas occur with equal frequency in both sexes and are described at all ages, with increased incidence in the 3rd decade. They vary in size (up to that of a bean) and are found chiefly in the tail of the pancreas. Multiple adenomas are not uncommon, but are usually so small that they cannot be palpated during operation. Adenomas may undergo malignant transformation, and insulin-producing metastases, especially in the liver, may occur.

Insulin-producing carcinomas of the islet cells, in contrast to the adenomas, are often quite large and may resemble sarcomas. Skiller and his coworkers have described 2 giant spindle-cell carcinomas of the pancreatic islands.

Diffuse hyperplasia of the islet tissue, without tumor formation, may occur in some cases of hyperinsulinism. Here the β -cells may be absolutely reduced in number or even absent.

Patients with diabetes mellitus often show adenomas, and some patients have been described who first showed hypoglycemia and later developed frank diabetes.

Classically, patients with hyperinsulinism show the same findings as seen in insulin overdosage, except that they have a prolonged, progressive course. The occurrence of a given episode is not necessarily correlated with a certain level of blood sugar. Characteristically, the most severe episodes take place during the night or before breakfast. The patient complains of earlier onset hunger, progressive muscular weakness, tremor, tachycardia, hot flashes, sweating, and nausea. Periodical paroxysmal stimulus vagus perverts may be present. Central nervous system symptoms include impaired speech, syncope, tonic and clonic convulsions or even paralysis, asthenia in bladder and rectal control, and unaccountable movements of the extremities (tremor, athetosis). Diencephalic signs include increased gastric secretion, elevation of temperature, disturbances of vision and hearing, and collapse. Psychic changes include clouding of the sensorium and coma, and there may be dream states, episodes of mania, and depression. Transient neurologic signs may suggest temporary involvement of the brain stem and the pyramidal tracts. The histopathology of such cases and the permanent damage due to therapy-resistant shock (Keymolen) have been discussed on p. 304 (Höpker, Erblich).

The diagnosis of hyperinsulinism is often delayed. Initially, the patient is usually suspected of having a brain tumor, epilepsy, or psychotic symptoms. The past history, however, and the clinical picture should alert the physician to the possibility of hyperinsulinism. The fasting blood sugar is often between 20 mg% and 40 mg%, a history of repeated loss of consciousness, especially during the night or in the early morning, is especially suggestive. Some cases report short, sporadic episodes related to meals. Wilder has recommended his "hunger test" for diagnosis, which regularly produces hypoglycemia in patients with hyperinsulinism, but has no effect in cases of neurogenic hypoglycemia. Tests of pancreatic function are of little value. Tests of glucose tolerance vary, sometimes showing diabetic curves, at other times severe hypoglycemia. The same variability is seen in tests which utilize epinephrine, insulin, and as a last resort the use of insulin tests because of the possibility of fatal hypoglycemic reactions. The electrocardiogram may show ST depression, flat or negative T waves, increased QT time - all due to hypokalemia, which is always present in severe hypoglycemia.

Therapy. The acute episode requires the immediate administration of carbohydrates by mouth or parenterally. Some authors report that L-glutamine acid intravenously corrects the loss of consciousness in most cases of hypoglycemic shock (Lager Gross and Walzer). Various other drugs have also been used, with varying results. Thus, intravenous alcohol in a dose of 150 to 250 mg/kg body weight had no effect, and in larger doses destroyed the β -cells of the pancreas. Improvement has been reported with thyroxin, anterior pituitary extract, and caffeine. Estrogen has had varying and unpredictable effects. Kirsch has pointed out multiple convulsions during pregnancy and has attributed the result to increased activity of the pituitary gland during pregnancy. In long term therapy, different diets have been recommended, varying from Com's low-carbohydrate, high protein (120 to 15 grams per day), moderate-fat diet, to Porter's diet which contains large amounts of carbohydrates.

Surgery, however, is the only satisfactory therapy in patients with progressive hyperinsulinism. Patients who are not treated die within a matter of years, but surgery has been reported to be successful in 70% of the cases. If no tumor can be palpated at the time of operation, partial pancreatectomy is indicated, with removal of two-thirds to three quarters of the pancreas (de Peyster and Gilchrist). The same operation is indicated in diffuse hyperplasia of islet tissue. In cases of pancreatic carcinoma, however, total pancreatectomy is indicated. Certain cases have been described in which severe psychic and nervous disturbances have persisted after successful operation; early diagnosis and therapy may prevent such sequelae.

Glycogen-Storage Diseases ("Glycogenoses")*

In 1929, von Gierke described a disorder characterized by increased storage of glycogen in the liver and in the kidneys. More recently, other disorders of glycogen storage have been described which can be differentiated from von Gierke's disease and from each other by clinical, pathological, and biochemical studies (Table 61). Andersen and G. Cori have distinguished 4 types: (I) Hepatorenal glycogen storage disease or von Gierke's disease; (II) Glycogen storage disease affecting the heart; (III) Diffuse glycogen-storage disease with cirrhosis of the liver; and (IV) "Benign" glycogen-storage involving the liver and the muscles.

Table 61. Classification of the Glycogen-Storage Diseases (according to Recant)

Type	I	II	III	IV
Tissues affected	Liver, kidneys	Cardiac and skeletal muscle, tongue, brain, often generalized	Liver (cirrhosis), reticuloendothelial system	Liver, muscle
Age at onset	Newborn, Infancy	Newborn, Infancy	Late infancy	Late infancy
Age at death	Varies according to severity of the disease	In the first 8 months of life	1-10 years	?
Hypoglycemia, Ketosis, Hyperlipemia	Very severe	Absent	Absent	Moderate
Reaction to epinephrine	Slight or none	Normal	Slight and prolonged	Slight
Structure of the glycogen	Normal	Normal	Abnormally long side chains	Abnormally short side chains
Enzyme changes	Glucose-6-phosphatase is diminished or completely absent	None	Probable diminution in the activity of "branching" enzyme system	Probable diminution in the activity of "debranching" enzyme system

* The sections on Glycogen-Storage Disease and Galactosemia have been revised by Dr. Nepomuk Zöllner.

This is a rare congenital anomaly of metabolism, recessively inherited, occurring exclusively in children and often manifest as early as infancy.

$$\text{glucose} \xrightarrow[\text{ATP}]{\text{hexokinase}} \text{glucose-6-phosphate} \xrightarrow{\text{glucose-6-phosphatase}} \text{glucose} + \text{P}$$

$$\begin{array}{c} \text{glycogen} \\ \updownarrow \\ \text{glucose-1-phosphate} \\ \updownarrow \\ \text{glucose-6-phosphate} \\ \updownarrow \\ \text{glycolysis} \end{array}$$

anomalous enzymatic cell structure \longrightarrow disturbed metabolism
 \longrightarrow storage \longrightarrow alteration of the cell and of the tissue structure.

The disease begins at birth or in infancy with hepatomegaly, which is often asymptomatic. The spleen is not enlarged, an important diagnostic point. The clinical picture is determined by the deficiency of utilizable sugars. Prodromal symptoms include a protruding abdomen, anorexia, loss of weight, vomiting, hypoglycemic convulsions, and rarely coma. As the disorder progresses, the child shows impaired growth and obesity, usually with mental retardation.

Objectively, the chief finding is the marked enlargement of the liver but not the spleen. The kidneys are enlarged and may be palpable. Because of impaired glycolysis, the blood sugar is low, and spontaneous hypoglycemia is common. The glucose tolerance curve is diabetic in nature, because of slow entry of glucose into metabolism. Epinephrine produces hyperglycemia only slowly if at all, although the blood pressure rises as usual. The blood sugar also fails to rise after the administration of glucagon. The status of metabolism may thus be likened to the tortures of Tantalus: in the midst of a surplus of carbohydrate, the patient lacks the means to utilize sugar. There is marked sensitivity to insulin.

The deficiency of utilizable carbohydrates is confirmed by the other laboratory tests. The blood shows increased amounts of ketone bodies, and acetone appears in the urine. Hyperlipemia is present, including an increase of cholesterol and of neutral fats. The serum phosphorus is usually reduced, and the liver function tests are usually normal.

The prognosis of von Gierke's disease is poor. Most children die of intercurrent infection, although a few reach adulthood, and spontaneous remissions have been reported. In very rare cases, true diabetes mellitus may occur later.

Therapy aims to supply energy by means of high-protein, low-fat, low-carbohydrate diets. Most authors warn against the use of hormones, including ACTH (van Crefeld), thyroid preparations, and pituitary implants (Bohlau), although a few authors use ACTH and cortisone to counteract hypoglycemia (Ulstrom et al.).

Pathologically, deposition of glycogen in the parenchymal cells of the liver causes the hepatomegaly, and in the renal tubules causes the enlargement of the kidneys. In addition to glycogen, the liver cells often show increased fat. The pancreas shows an increase of the B cells. The adrenal glands show hypertrophy of the medulla. These latter changes are probably secondary in nature. Biochemically, Cori and Cori found marked diminution of glucose-6-phosphatase in all 7 of their severe cases, as discussed above. In 5 less severe cases, the activity of the enzyme showed lesser reduction, and 1 mild case showed no diminution at all. The glycogen of all their cases was normal in structure.

Certain genetic relationships exist between glycogen-storage disease and diabetes mellitus. In Mauriac's syndrome, as already discussed (p. 316), both disorders are present in the same individual.

Rare Forms of Glycogen-Storage Disease

Type II: Cardiac Type.

The manifestations of this form begin in infancy with cardiorespiratory or neurologic symptoms. These include intermittent cyanosis, dyspnea, nutritional difficulties, and apathy. The picture may be mistaken for amyotonia congenita or for mongolism, and the enlarged tongue may suggest hypothyroidism.

The chief finding is marked enlargement of the heart, which may weigh 5 times the normal or more. The electrocardiogram shows negative T waves, depressed ST segments, and left axis deviation. Chemical studies give no clue to disturbed carbohydrate metabolism, and the diagnosis depends on muscle biopsy. Therapy is of no use, and the infant invariably dies in the first 8 months of life, usually of cardiac failure or intercurrent infection.

At autopsy, the chief finding is deposition of glycogen in the muscle of the heart. The striated musculature is also affected, especially the tongue and the diaphragm. The smooth muscles are also involved, more rarely the reticulo-endothelial system and the epithelial cells of the pancreas and the glands of the intestinal tract. Glycogen is also sometimes found in the sympathelic ganglia and in the cells of the central nervous system. No proliferative reaction is present in association with the glycogen storage. Biochemically, it has not been possible to demonstrate any abnormality of enzyme structure or of the glycogen of the affected tissues. The neuromuscular form of glycogen storage disease described by Selberg show muscular atrophy, and probably belongs best to type II.

Type III: Diffuse Glycogenosis with Cirrhosis of the Liver.

This is also known as "glycogen storage disease of the reticulo-endothelial system." The symptoms first occur towards the end of the first year of life or even later, and are all due to involvement of the liver: enlargement of the abdomen, edema, hemorrhagic tendency. Clinically, both hepatomegaly and splenomegaly are present, and ascites, jaundice, and anemia are present. The laboratory findings also reflect hepatic damage. Thus, the blood glucose is low, the glucose tolerance curve is flat and prolonged, and epinephrine produces a slight and prolonged rise of blood glucose. Therapy is symptomatic, and is directed against the cirrhosis, the hemorrhagic tendency, and the anemia. The patient invariably dies before the age of 10 years.

Pathologically, there is a fine, nodular cirrhosis of the liver with deposition of glycogen in the parenchymal cells. In addition, glycogen is found in the reticulo-endothelial tissue of the spleen and the lymph nodes. In contrast to types I and II, connective tissue proliferation occurs at the sites of glycogen deposition. Biochemically, the glycogen found is an abnormal glycogen which differs from normal glycogen by a smaller number of branches. This difference may be due to a lack of a "branching" enzyme in these patients. This pathological glycogen probably has the effect of a foreign body.

Type IV: Benign Glycogenosis of Liver and Muscles.

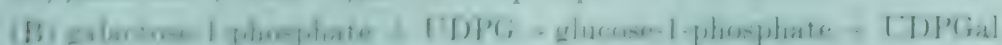
Only 2 cases of this variety of glycogen storage disease have been described to date. They are very similar to cases of von Gierke's disease (type I), but the distribution of glycogen is different: the muscles are especially affected. One of the 2 cases is still alive and is now 13 years old. He is a normally developed, healthy appearing child, but shows hepatomegaly with retention of bromsulfalein, hypoglycemia, diminished reaction to epinephrine, acetonuria, and hyperlipemia.

Biochemically, biopsies have shown a glycogen which contains an increased number of side chains. These side chains are very short. The reduced utilizability of the carbohydrate depots in these cases may be explained by the postulate that the breakdown of the glycogen is inhibited at the sites of branching. Since the side chains are very short, these sites are rapidly reached in metabolism, and the inhibition is explained by assuming reduced activity of an enzyme system which affects the glycogen at the sites of branching ("debrancher enzyme").

Galactosemia

Galactosemia is a congenital, hereditary disorder in which an abnormal metabolite of galactose seems to cause the development of hepatomegaly with jaundice and, eventually, ascites, proteinuria, aminoaciduria, cataracts, and retardation of mental development. In early cases, there is hepatocellular degeneration and bile stasis; in late cases, the liver shows fibrosis.

The basic metabolic defect is a lack of the enzyme phospho-galactose-uridylyl-transferase, which catalyzes reaction B in the following schema of the conversion of galactose to glucose:



The block of reaction B explains how endogenous galactose can still be produced (by reaction C) in spite of inhibited utilization of exogenous galactose. There is thus no deficiency of galactose which would decrease the synthesis of galactose-containing vital compounds such as the cerebroside and mucopolysaccharides. On the other hand, there is a marked increase of galactose-1-phosphate (e. g., in the blood), and this compound is probably the toxic substance in the disease.

The chief finding in galactosemia is hepatomegaly. The patient has recurrent episodes of vomiting and loses weight. Jaundice develops and increases in severity. The urine contains a reducing sugar whose presence in the early days of life should suggest the diagnosis of galactosemia. The sugar is, of course, galactose, and can be identified by such means as paper chromatography. Galactosemia, proteinuria, and aminoaciduria are also present. Cataracts develop as the disease progresses and may even be the presenting complaint.

Therapy consists of the rigid exclusion of galactose- and lactose-containing foods from the diet, i. e., milk, milk products, soy bean products, and certain "milk substitutes," as well as any tablet medications which contain lactose. When such a diet is followed, the children recover as far as possible and, when reversible damage is present, begin to develop normally.

The success of a galactose-free diet teaches an important general lesson. Galactosemia is a hereditary enzymic defect, an inborn error of metabolism. If galactose is omitted from the diet of children with the defect of galactosemia, they develop normally, while if galactose is fed they develop the characteristic abnormalities of the disorder. Thus, galactose, an environmental factor, induces the manifestations of the disease, so that if we knew less about the disorder we might consider it as galactose poisoning, an entirely exogenous disease. In reality, of course, it is an endogenous disease par excellence. The environmental galactose is the factor which induces a genetically predetermined disease, showing that a disease may be genetically determined as well as environmentally induced. This is obvious in galactosemia, but in other diseases where the causative role of environmental factors seems to be proven the situation may be basically similar to that in galactosemia.

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CHAPTER V.

Metabolism of Proteins and Amino Acids: Theoretical Section

By Kurt Felix *

Basically, the metabolism of proteins is the metabolism of their component amino acids, for most proteins are broken down to amino acids during digestion and, with the possible exception of a few polypeptides, it is the free amino acids which are absorbed and which enter into metabolism. The absorbed amino acids are distributed among the various body tissues, where some enter directly into metabolic reactions and are catabolized. Others are first transformed into tissue proteins and do not enter the metabolic processes until some later time. In addition, the tissues always contain small amounts of free amino acids derived partly from the ingested food and partly from the catabolism of tissue proteins.

The constant stream of nitrogenous material which flows through animal and human organisms is supplied by the proteins of ingested vegetable and animal food. Protein metabolism is altered in many disease states, usually quantitatively, but occasionally qualitative changes also occur. The quantitative changes manifest themselves as an imbalance of protein metabolism.

Protein balance is usually measured by measuring nitrogen. Nitrogen balance studies reveal nothing, however, about carbon and sulfur balances. The metabolic pathways of these three important elements, which are present in all natural mixtures of proteins, are separate from one another. Phosphoric acid, which is also present in certain proteins, also has its own separate fate. These facts were known to the older physiologists and have been reaffirmed in recent years by means of isotope research.

Nitrogen Balance

Nitrogen balance is determined by comparing the nitrogen taken into the body in the form of food protein with that excreted in the urine and stool as nitrogenous waste products. (Except in certain special cases [see page 333], the nitrogen lost in hair, sweat, scales, saliva, and similar body products can be ignored.) Nitrogen balance can be neutral, positive, or negative. In neutral nitrogen balance, as much nitrogen is excreted as is taken in; in positive nitrogen balance, more nitrogen is ingested than is excreted; and in negative nitrogen balance, more nitrogen is excreted than is ingested.

* Prof. Felix died on August 2, 1960.

Nitrogen Equilibrium

When nutrition is normal, as much nitrogen is excreted as is ingested. This neutral nitrogen balance is found with both very small and very large amounts of protein ingestion.

On the average, man excretes approximately 1.3 grams of nitrogen in the stool and 13 grams of nitrogen in the urine per day. The nitrogen of the stool is derived from undigested and unabsorbed food, from the intestinal flora, and from secretions which are emptied into the intestinal tract.

When there is free choice of food, the protein intake and therefore the nitrogen balance depends, among various peoples and within a given population in various occupational groups, on many factors: the climate, the available or prescribed foods, the occupation, and the composition of the diet (content of fats, carbohydrates, vitamins, and inorganic salts).

Krogh reported that Eskimos consume 2 to 4.5 pounds (1-2 kg.) of lean meat daily. This amount contains 200 to 400 grams of protein. The same is true of the Caucasian shepherds. The rural people of central Germany also consume a relatively large amount of protein daily (120 to 150 grams). On the other hand there are occupational groups in Germany and in certain sections of India which get along on 30 to 40 grams of protein per day. During the blockade of Germany, the daily ration of protein was reduced to 30 to 60 grams. The less protein in the diet, the more carefully the component foods must be chosen.

Röse formulated a diet for himself which contained only 25 grams of protein per day. He stayed on this diet for years and was able to maintain his health and to continue his activities as a physician. However, every time he contracted a digestive upset or a cold, his nitrogen balance became negative and he found it necessary to interrupt his diet with a few days of protein-rich food.

When one changes experimentally from one type of nitrogen equilibrium to another, the new equilibrium does not assert itself immediately. There are several days in which the balance is either negative or positive, becoming negative when one goes from a higher to a lower balance and positive when one passes from a lower to a higher balance. In the latter case, protein is retained in some form in which it remains readily available and can be easily utilized when circumstances necessitate. When protein is completely omitted in animal experiments, the animals excrete more nitrogen the greater their weight and the higher their previous nitrogen balance. The ability to retain protein is not equally developed in all individuals, and the type of protein in the food itself seems to have a distinct influence on this ability. Thus, animal protein can be retained somewhat more easily than plant protein. Casein is not a good precursor of storage protein.

Probably nearly all body tissues are capable of storing protein, especially the liver, heart, kidneys, and the skeletal muscles. The brain and spinal cord evidently do not store protein. The form in which protein is stored is poorly understood; certainly it is not as free amino acids and not as a storage material similar to that of plant seed protein. Probably, each tissue merely synthesizes more of its own particular protein. In favor of this hypothesis is the fact that the size of the liver increases in animals which are fed diets rich in protein without any corresponding alteration in the percentage composition of the organ; i. e., the content of nitrogen and amino acids remains unchanged.

When an experimental animal (rat) is fed a protein-rich diet, the cytoplasm of the liver cells develops numerous granules which can be stained with pyronine. Starving

the animal then causes these granules to disappear. These granules are derived from reticuloeproteins and comprise a special form of storage protein [1]. This storage protein is thought to be important in the ability of the organism to resist infection and other illnesses, since it is generally held that the body nourished with protein-rich food is better able to produce antibodies than that which receives protein-poor food and that which is starved.

Nitrogen balance is normally positive in growing children, in patients during convalescence from wasting diseases, and in pregnant women. Nitrogen balance is negative only under certain special circumstances such as starvation, malnutrition, febrile illnesses, burns, wounds, and abdominal operations.

The upper limit of protein intake at which balance can still be maintained is not sharply demarcated but depends on the digestive power of the gastrointestinal tract. The lower limit, on the other hand, depends on the conditions of endogenous metabolism and the properties of the food proteins.

As long as there is life, protein is always being metabolized. It must therefore be taken in from the outside in order to maintain equilibrium. The minimal protein metabolism can be attained by eliminating all protein from the diet for several days, supplying the energy requirements with carbohydrates and fat, and providing an adequate supply of vitamins and minerals. Under these circumstances, the daily excretion of nitrogen falls to 1.7 to 3 grams, which corresponds to a protein metabolism of 9.4 to 19.0 grams [2]. A full-grown man metabolizes 0.156 to 0.35 grams of protein per kilogram of body weight per day [3]. Cathcart was able to reduce the excretion of nitrogen to 2.84 grams per day [2]. The lowest value reported was obtained by Boothby and Sandiford, who recorded 1.74 grams per day, which corresponds to 0.024 grams of nitrogen per kilogram body weight [3]. M. Rubner calls this minimal metabolism the "wear-and-tear portion." It includes the protein losses of the intestinal secretions; the desquamations of skin, kidneys, bladder, and intestine; and those end products of metabolism which can only be attributed to proteins. Included also are the syntheses of protein hormones (insulin, glucagon, pituitary hormone, parathyroid hormone) and proteogenic hormones (epinephrine, thyroxine), as well as their conversion into inactive and easily excreted compounds. Under the conditions of minimum endogenous protein, all other metabolic requirements are supplied by the other constituents of the diet.

The endogenous minimum of protein is a measure of the protein which is broken down into the excretory products under the conditions described. There is also another internal cycle in which amino acids and peptides liberated in one portion of a cell or tissue or organism are again built up in another portion. The extent of these reactions is not precisely known, but an approximate conclusion can be reached by experiments with amino acids tagged with isotopes. K. Lang, using the data of various authors, calculated that an adult man synthesizes 98 grams of protein daily [4], partly from the amino acids in food and partly from amino acids liberated in intermediary metabolism. This value corresponds to a daily nitrogen turnover of 15.7 grams. The endogenous minimum is only a fraction of this figure, and it is not known if this much protein is newly synthesized each day under the minimum conditions.

The amount of nitrogen excreted during minimum protein metabolism depends on a number of factors, one of which is the size of the organism. Calculation of the nitrogen excretion per kilogram of body weight discloses that smaller animals excrete a proportionally larger amount of nitrogen. In other words, the level of the endogenous

minimum protein metabolism is greater in smaller animals than in larger ones [5]. The same result was found by Sprinson and Rittenberg [6] in experiments with N^{15} -labelled glycine: the rat turned over per kilogram of body weight about five times more protein nitrogen than man.

Environmental temperature also influences the nitrogen minimum. In general, the higher the temperature, the less nitrogen is excreted [7]. The endogenous nitrogen minimum resembles the basal metabolism in this respect: when both are calculated per kilogram of body weight, the resulting value is nearly constant at various temperatures. Thus, Terroine and Sorg-Matter found in the mouse that at 25° the excretion of nitrogen was 0.0342 grams/kg./hour and at 30° it was 0.02695.

It is doubtful if muscular activity influences the endogenous nitrogen minimum [8]. On the other hand, there is a relationship between protein metabolism and the body content of minerals. Wherever protein is synthesized, various salts are retained which are necessary to provide the protein with the required degree of hydration. Withholding salts from an animal thus impairs its growth [9]. A full-grown animal metabolizes more protein during salt-deficiency than while on a salt-containing diet [10]. An excess of salt, however, is also deleterious [11]. The effect of salt depletion on protein metabolism is probably indirect, by way of the effect on pH. When the amount of available base is insufficient, more ammonia must be produced by deamination for the neutralization of the acids formed in metabolism [12]. Furthermore, the protein balance of tissues becomes negative when the tissues lose phosphorus and potassium.

Biological Value of Proteins

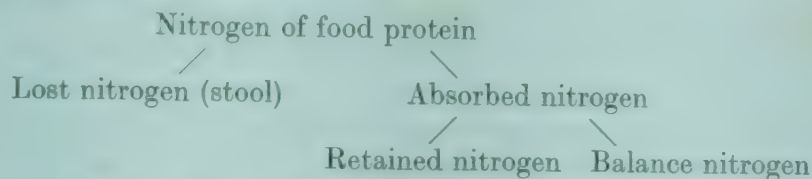
When protein is ingested, nitrogen excretion in the urine usually rises. For this reason, it is only under certain circumstances that it is possible to equalize the nitrogen balance with the amount of protein which corresponds to the endogenous nitrogen minimum, and usually more than the calculated amount of protein must be supplied. With many proteins, it is impossible to attain equilibrium because they lack one or another essential amino acid. Thus, gelatine lacks tryptophane; gliadin lacks glycine; and so on.

If all essential amino acids are present, the level of equilibrium reached depends on their relative proportions in the diet. The lower this level, the higher is the "biological value" of the proteins involved. Usually, however, the biological value of a protein is arrived at in a different way: the term signifies the percentage of absorbed protein nitrogen in food which is retained within the body and is therefore presumably converted to tissue protein.

The concept of *biological value* was suggested by K. Thomas [13]. He placed his experimental animal or human subject on the endogenous nitrogen minimum for a period of one week and measured the daily excretion of nitrogen in the urine and stool. The urinary excretion of nitrogen reflected the endogenous minimum and the nitrogen in the stool reflected the nitrogen-containing constituents of gastric, pancreatic, intestinal, and biliary secretions which were not absorbed. The stool value also included desquamated intestinal mucosal cells and bacteria. (The cells of the intestinal mucosa have only a limited life and are rapidly replaced by new cells. A cell of the duodenal mucosa lives an average of 1.57 days, and one from the mucosa of the ileum only 1.35 days [13 a]). In the following week, Thomas gave a diet which contained 8 to 10% protein and again measured the daily output of nitrogen in the urine and the stool. There was an increase in the urinary nitrogen as a result of non-retained

dietary nitrogen. The difference between the urinary nitrogen during the protein period and the endogenous minimum was designated "balance nitrogen." The stool nitrogen during the period of protein ingestion consisted of nitrogen derived from bacteria and bodily secretions, plus that part of the ingested nitrogen which was not absorbed into the body. The stool nitrogen was called "lost nitrogen" or "unavailable nitrogen." Thomas assumed that exactly the same amount of nitrogen was secreted during the protein period as during the protein-free period, and Mitchell and Best have recently proven the correctness of this assumption [14].

If one subtracts this lost nitrogen from the protein nitrogen of the food, the result is a measure of the amount of nitrogen absorbed into the body. The difference between the absorbed nitrogen and balance nitrogen gives the retained nitrogen. The following schema explains the various nitrogen fractions:



The biological value BV can then be derived from the following formula:

$$\text{BV} = \frac{\text{retained N} \times 100}{\text{absorbed N.}}$$

This method of calculating the biological value of proteins involves a number of uncertainties besides the obvious uncertainty of the amount of nitrogen due to bacteria and body secretions. During the protein period the amount of nitrogen retained changes during the course of the week. At first, it is relatively large, later becoming smaller and smaller, so that, in other words, the biological value is high at first and then becomes lower and lower. This is due to the fact that, in the preceding week, the protein reserves were depleted and are now again becoming filled.

Finally, the biological value varies with the proportion of protein in the diet. The higher this proportion, the lower is the biological value of the diet.

These errors can be avoided when even less protein is given to the subject, so that the nitrogen balance becomes negative [15, 16, 17]. This occurs when there is 3 to 4% protein in the diet. Under these conditions, there is a direct proportion between the amount of absorbed protein nitrogen and the amount of nitrogen in the urine. Endogenous nitrogen and balance nitrogen then make up the urinary nitrogen, as follows:

$$\text{urinary N} = \text{endogenous N} + \text{balance N.}$$

In accordance with the schema above, balance nitrogen equals absorbed nitrogen minus retained nitrogen. During negative nitrogen balance, the retained nitrogen is equal to the absorbed nitrogen multiplied by a constant K. The equation above can then be rewritten as follows:

$$\text{urinary N} = \text{endogenous N} + (1-K) (\text{absorbed N})$$

In negative nitrogen balance, this equation represents a straight line with a slope of (1-K). If K = 1, the slope becomes zero; that is, only endogenous nitrogen and no balance nitrogen is excreted. In other words, the total absorbed nitrogen is retained in the body under these conditions. The value of the biological value factor is then 100. In rat experiments, the proteins of chicken egg approach this value: the value of K is 0.99 and the biological value of this protein for the rat is therefore 100×0.99 , or 99.

In most cases K is less than 1. When half of the absorbed nitrogen is excreted as balance nitrogen, K is 0.5 and the biological value is 50. For milk protein, K is 0.857, and the biological value is 86. For soya bean protein, K is 0.494, and the biological value is 49.

Excellent proteins are found in chicken egg, milk, kidney, and liver. Good proteins are found in animal muscle, fish, peanuts, and potatoes. Less good protein is present in corn and edible roots, and the poorest is that in most nuts and vegetables.

The error of calculation due to reduced protein reserve is not entirely eliminated by this method of determining the biological value of a protein. A number of workers therefore attain the endogenous minimum, not by means of a protein free diet, but by a diet which contains sufficient egg protein (albumin) to maintain a negative balance. Under these conditions, all absorbed nitrogen is retained, the reserves are full, and the protein under study is being investigated under conditions which approach the normal.

The biological value of a protein depends on the amino acids which it contains. The most important of these are the *essential amino acids*, i. e., those which cannot be synthesized by the body. The first amino acids which were recognized as essential were lysine and tryptophane. Osborne and Mendel gave growing rats a diet consisting of highly purified substances; the rats stopped growing whenever the protein in the diet lacked one of these two amino acids and began to grow again when the amino acids were added to the diet. In various experiments with carefully purified preparations, W. C. Rose [18] showed that eight of the known amino acids are essential for the adult and two others are essential for the growing animal. The first eight are lysine, tryptophane, phenylalanine, threonine, valine, methionine, leucine, and isoleucine. The two additional ones are arginine and histidine, which the growing organism is incapable of synthesizing in sufficient amounts for growth.

Rose found that he was able to maintain the adult man in nitrogen balance with these eight amino acids, provided that additional nitrogen (in the form of ammonium salts or glycine), carbohydrates, and carboxylic acids were included in order to allow synthesis of the missing amino acids. As soon as one of the essential amino acids was omitted from the diet, nitrogen balance became negative. When the missing amino acid was replaced, nitrogen balance again corrected itself [19, 20, 21]. The daily minimum and optimum requirements of these amino acids for the adult are given in Table 62 [22].

Table 62. Daily Requirement of Amino Acids in the Adult

	Minimum Requirement G/day	Optimal Requirement G/day
L-tryptophane	0.25	0.5
L-phenylalanine	1.10	2.2
L-lysine	0.80	1.6
L-threonine	0.50	1.0
L-valine	0.80	1.6
L-methionine	1.10	2.2
L-leucine	1.10	2.2
L-isoleucine	0.70	1.4

The statement that, of all known amino acids, ten are "essential," does not mean that the other amino acids are without value. The others can be omitted from the diet only in experimental studies and not in normal nutrition. They are found in all tissue proteins as building blocks and have additional important functions. Alanine, aspartic

acid, and glutamic acid support the citric acid cycle and are necessary for transamination. Many amino acids can also be converted to glucose ("glucoplastic" amino acids). Tissues cannot be nurtured with the essential amino acids alone, and when the other amino acids are lacking in the diet, the body is forced to function under unnecessary handicaps.

If the biological value of a protein depended solely on its content of amino acids, especially the essential amino acids, it would be possible at least to approach the calculation of biological value by studying the composition of the protein. In order to do this, one needs a protein with an optimal mixture of amino acids. The protein of egg has a biological value approaching 100 and is therefore excellent for such a study. The composition of the protein under investigation is compared with that of egg albumin and the "limiting" essential amino acid is determined - i. e., that whose concentration is furthest from that of egg albumin. In most proteins - cheese, beef muscle, kidney, peas, etc. - this amino acid is methionine. In many other proteins (sunflowers, flax seeds, and others) it is lysine. In a few (hemoglobin, serum protein, liver protein), it is isoleucine. In gelatin, it is tryptophane.

The deficit in the limiting essential amino acid expressed in percent and subtracted from 100 represents the approximate biological value [23, 23 a]. In soya bean protein, there is a deficit of 51% in methionine, from which a biological value of 49 can be calculated; this is the value actually found in balance studies. Liver protein contains 30% too little of isoleucine, giving a calculated biological value of 70; the actual value from balance studies is 77. In other experiments, the correlation between the results found by the two methods is less good, as judged by chicken experiments.

In order that essential amino acids be utilized for the synthesis of tissue protein, they must all be present at the same time [24] (p. 351). When one or another of the amino acids is liberated too slowly during digestion, or is absorbed too slowly, this simultaneity is not attained and a poorer biological value results [23, 25]. The splitting of proteins by the digestive enzymes is aided by proper preparation of the food. Experiments with soya beans have shown that the enzymic action is aided by warming, but inhibited by prolonged heating [26, 27]. When a protein is overheated, lysine is liberated more slowly and in lesser amounts by digestive enzymes [23].

The value of a protein can be increased by the addition of those essential acids which are present in too low concentrations. It can also be increased through suitable mixtures of proteins. When a certain group of proteins is to be replaced in the body, the biological value of the food protein changes, and it is best to supply those proteins whose structures come closest to the protein to be synthesized. Serum protein is thus a good substitute for plasma protein.

In addition to the ability to maintain nitrogen balance, biological value can be measured by the ability to promote growth. The weight gain which results from the utilization of one gram of protein nitrogen is determined in a young animal in this technic. This method measures the ability of a protein both to maintain weight and to promote growth. Egg protein is again superior in this respect to the proteins of wheat and soya bean [15].

Nitrogen Balance by Mixtures of Amino Acids

With the finding that maintenance of balance and growth depends on the amino acids present in protein and to a lesser extent on the manner in which they are bound together, the next step was to use mixtures of amino acids in nutrition. E. Abderhalden was the first to employ this technic of study. He maintained a dog in nitrogen

balance for several months by means of a mixture of pure amino acids such as that found in beef muscle [29]. Similar experiments were later repeated, chiefly by W. C. Rose. The results of these experiments was the list of essential amino acids.

The clinical use of amino acid mixtures in nutrition is limited to special situations. Undernourished, markedly weakened, and postoperative patients can be rapidly restored to normal strength by such mixtures without burdening the intestinal tract. Such mixtures of amino acids can actually be used to supply the entire protein requirement by the intravenous route. The body is said to use up more energy to maintain nitrogen balance under these conditions than when protein is supplied in the usual way in the diet [22, 30].

Since intravenous administration of amino acids may be accompanied by chills and other reactions, it is preferable to give the amino acid mixtures by mouth. The oral route has the additional advantage of better assimilation: intravenous administration is followed by a greater loss of amino acids in the urine than oral ingestion. In addition, the utilization of the free amino acids given by vein is not entirely complete: thus, in order to supply the needs for phenylalanine, more must be administered by vein than if given by mouth [31]. The amino acids must first pass through the liver, apparently, in order to be directed into the proper metabolic pathways. Some proteins, such as homologous plasma protein of man [32] and dogs [33] are better utilized in nitrogen balance when given intravenously than by mouth. When these proteins are given by mouth, their nitrogen appears in the urine after one day, while after intravenous administration only a third of the material appears in the urine during the next few days and the remainder does not appear for some time (over 3 weeks).

Negative Nitrogen Balance

In addition to those instances in which, as a result of various restrictions or intentional experimental conditions, less nitrogen is taken in than is excreted, there are also certain situations in intermediary metabolism in which protein is broken down in increased amounts to such a degree that the balance can barely be maintained through food. This condition is found chiefly in pathological states and will be mentioned here only briefly. Negative nitrogen balance can be seen in febrile illnesses, burns, wounds, abdominal operations, severe hyperthyroidism, and carcinomatosis. Nitrogen balance also becomes negative when protein synthesis is impaired, as in decompensated liver cirrhosis and in atrophy of the liver, and this is especially true when ascites is present and abdominal paracentesis must be performed [28]. If negative nitrogen balance persists for a sufficiently long time, protein reserves are depleted and then the protein of the blood plasma, especially the albumin fraction, is depleted, the result being hypoproteinemia.

Digestion of Food Proteins

Breakdown in the Gastrointestinal Tract

The glands of the gastrointestinal tract secrete a series of proteolytic enzymes, proteases, which are capable of completely splitting the proteins of food into their component amino acids. The three proteinases are pepsin (in gastric juice) and trypsin and chymotrypsin (in pancreatic juice). These enzymes split peptide linkages within the protein chains and are therefore called "endopeptidases" [34, 35].

Pepsin acts on practically all proteins except protamines and keratins. It has a molecular weight of approximately 34,000, works best at pH of 1.5 to 2, and is denatured at pH 6 [34]. It acts chiefly on the peptide linkages, where the amino groups of tyrosine or phenylalanine are bound [36, 37, 38].

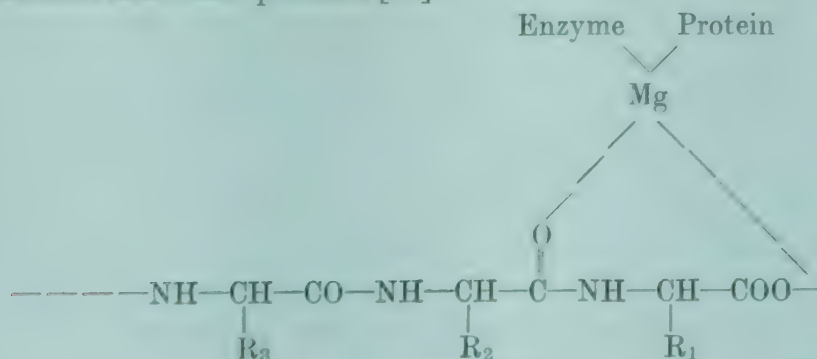
Trypsin is specific for the peptide bonds by which the carboxyl groups of basic amino acids are bound [39]. It also splits the esters of the basic amino acids, as for example benzoyl-L-arginine-methyl-ester; but not benzoyl-L-leucine-methyl-ester [40]. It has a molecular weight of about 36,000 and works best at a pH of 8, but can also function at higher concentrations of hydrogen ions down to pH 6. This fact is of importance, because the pH of the duodenal contents is usually weakly acid.

The second proteinase of pancreatic juice, chymotrypsin, has an optimum pH at 8 and preferentially hydrolyzes bonds whose carbonyl group is attached to a tyrosine rest. For tyrosine may be substituted phenylalanine, tryptophane, and methionine [41].

Chymotrypsin also splits esters, but only esters of the aromatic amino acids, especially tyrosine - e. g., benzoyl-L-tyrosine-ethyl-ester [42]. The enzyme is inhibited by fluoro-di-iso-propyl-phosphate, which apparently reacts with one of the serine residues of the enzyme molecule [43]. Chymotrypsin is not a homogeneous material, although its precursor, chymotrypsinogen, is the most homogeneous protein known. Three components of chymotrypsin have been separated, with molecular weights of 43,000, 30,000 and 27,000 [44].

Each proteinase by itself is not capable of splitting proteins to any great degree. When all 3 work after one another, however, there results a number of large or small polypeptides which are then further broken down by the two *polypeptidases*. Only rarely, if at all, do amino acids become free. Thus, lysine was found in pepsin hydrolysis of the histone of thymus [45]. Of further significance is the fact that the two pancreatic proteinases attack native proteins which have not previously been split by pepsin. For this reason, in people in whom the gastrointestinal tract no longer secretes pepsin or in patients with gastrectomy, the digestion of proteins is not noticeably impaired.

The *carboxyl polypeptidase* of pancreatic juice, molecular weight 34,300, splits off one amino acid after the other from the carboxyl end of the polypeptide. The kind of amino acid which is present at the carboxyl end has a definite effect on the ease with which splitting occurs. Phenylalanine is set free most rapidly, followed by tyrosine, tryptophane, leucine, methionine, isoleucine, alanine, and glycine [46]. Carboxylpolypeptidase also splits amino acids from the carboxyl end of the peptide chain in protamines and other proteins [47].



Besides this, there are other enzymes which have not yet been investigated [48]. Some of these hydrolyze only tripeptides; others work only on dipeptides.

Carboxyl polypeptidase requires for its action a free carboxyl group. Amino polypeptidase requires a free amino group next to the bond which is to be split.

The dipeptides of proline, in which the imino group is bound, are acted upon by a special dipeptidase called "prolidase." In addition to this, there probably are still other *dipeptidases*.

The poly- and di-peptidases are probably metalloproteins. Metallic ions such as Mn^{++} , Mg^{++} , Co^{++} , and Zn^{++} activate certain peptidases, whose action is said to depend on the fact that, under the influence of the metallic ion, they undergo chelation with the substrate which includes the bond which is to be split. The metal so deflects the electrons that the bond is opened at physiological concentrations of the hydrogen ion [49]. For the carboxypolypeptidases, a chelating mechanism is also suggested for the union of enzyme and substrate.

Absorption of Products of Protein Digestion

As is well known, absorption from the intestinal mucosa consists primarily of free amino acids, with perhaps a few peptides. As regards the peptides present in the portal blood, these need not be the result of digestion but could be synthesized in the intestinal mucosa [50, 51]. In a few individuals, unsplit proteins can also be absorbed and may cause allergic reactions. In the intestine of the infant, large molecules seem to be absorbed, for example the antibodies present in colostrum.

The exact mechanism of absorption of amino acids is not known. Just as little is known concerning whether and to what degree the amino acids are incorporated into the protein of the intestinal mucosa. Experiments with isotopes have shown that a few amino acids remain in the intestinal mucosa longer than others, and it is probable that these are incorporated into the protein of the mucosa.

The products of intestinal digestion of proteins are absorbed primarily in the small intestine, and practically not at all in the stomach and in the rectum (as shown by recent experiments in rabbits utilizing S^{35} -labelled proteins) [52]. Under normal conditions of food intake, these products go exclusively into the blood stream. It is only when very large amounts of protein are ingested that some of the absorbed protein digestive products also appear in the lymph. The lymph of the thoracic duct contains 2 to 4 percent of protein.

Experiments in dogs have shown that the content of free amino nitrogen in the blood of the mesenteric artery increases by 20% during digestion [53]. Theoretically, a greater increase might be expected, because the level of sugar in the portal blood rises much more, from 100 $mg\%$ to about 400 $mg\%$. Perhaps, therefore, some amino acids are transformed in the intestinal mucosa directly into protein, and then pass into the blood as such. A large fraction of the absorbed amino acids is retained in the liver. A small fraction goes through into the peripheral blood, where a portion of the amino acids remains in the plasma, and a portion passes into the red blood cells. The plasma of a fasting individual contains approximately 5.5 to 8 $mg\%$ of amino acid nitrogen; 5 hours after a meal, this figure becomes 7 to 10 $mg\%$; and 7 hours after this, it falls again to the fasting level [54]. Table 63 gives the plasma levels of a few of the free amino acids [55].

The concentrations of the individual amino acids depend on ratio in which they are absorbed. When only a single amino acid is administered, its concentration in the

plasma goes up, while that of other amino acids may fall. L-proline, L-histidine, L-methionine, D,L-threonine, glycine, D,L-serine, D,L-valine, and D,L-alanine cause a rise of the concentration of the blood amino acids. A large amount of glycine causes an increase of its own concentration, and a decrease of that of the others. Phenylalanine, tyrosine, tryptophane, and arginine have a lesser effect [56].

Table 63. The Concentration of Amino Acids in the Plasma of Normal Adult Men (after Harris)

Amino Acid	mg%
Aspartic acid	0.03
Asparagine	0.58
Glutamic acid	0.70
Glutamine	8.30
Glycine	1.54
Alanine	3.41
Amino-butyric acid	0.30
Valine	2.88
Leucine	1.69
Isoleucine	0.89
Serine	1.12
Threonine	1.39
Cysteine and Cystine	1.18
Methionine	0.38
Taurine	0.55
Proline	2.36
Phenylalanine	0.84
Tyrosine	1.03
Tryptophane	1.11
Histidine	1.15
1-Methyl-histidine	1.11
3-Methyl-histidine	0.08
Ornithine	0.72
Lysine	2.72
Arginine	1.51
Citrulline	0.50
β -amino-butyric acid and β -alanine	0.20

From the blood, the tissues of the body rapidly take up those amino acids which they need. In starved animals, the peripheral tissues, including the liver and the muscle, contain some 40 mg% of free amino nitrogen, which is some 10 times as much as is present in the blood. When amino acids are given intravenously, their concentration in the tissues increases $1\frac{1}{2}$ to 3 times (in the muscle, to 60 mg%; in the kidneys, to 80 mg%), and these values are maintained for the 3 hours following the infusion. In the liver, the level of amino acids rises to 120 to 160 mg% but falls rapidly to its original value, and at the same time there appears in the blood an equivalent amount of urea nitrogen [57]. Urea is formed chiefly in the liver (in mammals, exclusively in the liver). Hepatectomized dogs no longer synthesize urea, and the blood levels of the amino acids rise [58].

In normal man the liver contains 37.1 mg% of amino nitrogen, the kidneys 44 mg%, the muscles 19.2 mg%, and the brain 43.1 mg%.

The level of amino acids in the blood is influenced not only by the amount of protein in the diet, but also by certain hormones. Insulin and epinephrine cause a

reduction in this level, while pituitary growth hormone and adrenocortical hormone cause an increase [57].

The concentration and type of free amino acids in the blood and tissues depend largely on the liver. When the liver is excised from dogs which have been allowed to starve for 20 hours before operation, and when enough glucose is administered to prevent hypoglycemia, the free amino acids of the plasma rise from 3.5 mg% to 8.5 mg%. Of these, glutamic acid, which is already present in high concentrations, rises the most, from a level of 0.88 mg% to a level of 2.9 mg% [59].

Free Amino Acids in the Tissues

As already described, the tissues contain from 5 to 10 times as much free amino acids as the plasma; they must therefore expend energy in order to produce this high concentration. Most of this energy they obtain through an oxidation process (perhaps of pyruvic acid, succinic acid, or α -ketoglutaric acid), because in the absence of oxygen the tissues can no longer concentrate amino acids. It is not yet known whether the energy required is also obtained from ATP or other high-energy compounds [60].

The uptake of amino acids by the cells depends primarily on their concentration in the extracellular fluid. For a given amino acid, if this concentration is lower than that in the plasma, this amino acid is not taken up by the cells. According to experiments in rats, the critical level for glycine is 0.456 mg%. The inorganic ions also seem to be involved in the process. Thus, removal or complex binding of calcium inhibits the uptake of the amino acids by the cells, and if sodium is replaced by potassium in the extracellular fluid, the cells lose free amino acids.

Amino acids also influence each other. When the concentration of one of the amino acids is increased in the plasma by administration of this amino acid (for example, L-proline, L-histidine, glycine, L-methionine, D,L-threonine, D,L-serine, D,L-valine, and D,L-alanine), then the muscle and liver cells take up more of it. However, the intracellular concentration of other amino acids may be reduced by this process. If, on the other hand, the concentration of one of the amino acids in the blood plasma is reduced, the tissues do not take up this amino acid; but, over and above this, they also take up less of other amino acids. When rats are fed a diet which is poor in methionine, not only do the liver and brain content of methionine fall, but their concentration of arginine also falls. In addition, the hepatic levels of isoleucine, leucine, and phenylalanine also tend to fall. Lysine, on the other hand, increases. In the brain, tryptophane and valine are reduced, but histidine is increased [61]. The liver and the brain thus react differently to the deficiency of methionine. Perhaps this is an indication that they require amino acids in different amounts, and their pathways of catabolism are intertwined in various ways.

Thus, the amino acids compete with one another in their entry into the cells [56]. Glutamic acid is an exception and seems to increase the absorption of other amino acids into the cells.

Finally, certain vitamins also have an effect on these events. The muscles of guinea pigs lose glycine and glutamine when there is a decrease in ascorbic acid [62]. Pyridoxal encourages the uptake of glycine [63].

The *free amino acids* which are already present *in the tissues* are apparently available for immediate use by the cells in time of need. They are taken up from the food by the blood to the degree to which they are required. If the dietary intake is inter-

rupted, there is a breakdown of cellular protein - that is, of the labile storage protein. Amino acids are thus an intermediate step between the protein of food and the protein of the tissues (p. 331).

Up to a certain point, the free amino acid composition of the body stores can be characteristic for the individual tissues because it is from the stores that the specific tissue proteins are built up and in the breakdown of these proteins the same amino acids are produced. So, one might expect that the composition of storage protein as regards free amino acids is the same as that of the tissue protein involved. However, since amino acids can also be oxidized and can, on the other hand, be newly formed from keto acids, the similarity need not be great.

In the liver, the composition of the stores of free amino acids seems to depend largely on the diet, as was shown by O. Wiss in rats [64] (Table 64). The total concentration of the amino acids changes very little, but their relative proportions change considerably. The same principle holds for human beings.

There are few observations of the free amino acid content of human tissues. On the other hand, the free amino acid content of body fluids and of tissues of a number of the higher and lower animals have been well investigated. The results afford an interesting view into the interaction between animal and environment in the field of protein metabolism. In order to give an idea of the order of magnitude of the results, Table 65 presents analyses of plasma and muscles of rats, rabbits, cats, and chickens according to Florkin [65]. An additional table (Table 66) presents the values for the free amino acids in the liver and brain of young rats.

The concentration of the free amino acids in the tissues depends, like that in the blood, on the presence of the liver. A chromatogram of the free amino acids in the cerebrum and cerebellum of the normal dog shows small spots corresponding to

Table 64. Concentration of amino acids in the liver of rats in mg% of the fresh weight. (After Wiss [64])

	Starvation	High-Carbo- hydrate Diet	High Protein Diet	High Fat Diet
Valine	12.3	5.1	10.7	6.1
Leucine	7.58	5.46	9.08	5.7
Isoleucine	5.6	3.38	7.72	4.27
Methionine	2.9	0.92	2.35	1.94
Phenyl-alanine	4.4	2.68	3.76	3.34
Histidine	6.6	8.5	7.8	7.1
Arginine	1.1	0.19	0.89	0.52
Lysine	14.1	8.3	11.6	10.1
Tryptophane	0.15	0.03	0.21	0.12
Threonine	11.6	5.1	8.9	6.7
Glycine	25.1	28.5	20.0	28.6
Alanine	49.4	109.0	65.5	57.7
Cystine	1.6	0.86	1.6	0.76
Serine	19.6	17.6	11.6	25.8
Aspartic acid	21.8	29.9	16.1	25.4
Glutamic acid	130.5	121.0	139.0	123.3
Tyrosine	4.25	2.36	2.84	2.87
Proline	6.06	4.0	6.65	4.88
Hydroxyproline	1.7	0.47	1.4	0.58
	326.43	353.35	327.7	315.78

Table 65. The non-protein-bound amino acid content of the muscles and plasma of rats, rabbits, cats, and roosters. Data from the literature.
(Mg. per 100 grams of fresh tissue or per 100 cc. of plasma. NH = non-hydrolyzed filtrate or dialysate; H = hydrolyzed dialysate or filtrate.)

	Rats						Rabbits						Cats						Rooster					
	Plasma			Muscle			Plasma			Muscle			Plasma			Muscle			Plasma			Muscle		
	NH	H		NH	H		NH	H		NH	H		NH	H		NH	H		NH	H		NH	H	
Alanine	—	—	—	—	—	—	—	8.2	—	—	16.9	—	7.0	7.3	24.7	24.3	4.6	11.1	5.2					
Arginine	2.6	3.6	—	6.3	4.5	2.5	2.5	2.2	3.5	3.0	3.0	1.4	1.4	1.6	2.7	3.0	2.2	1.3	1.3					
Aspartic acid	—	—	—	—	—	0.0	0.0	1.7	2.5	7.2	7.2	0.1	0.1	1.4	3.9	9.8	3.4	2.2	3.2					
Glutamic acid	—	—	—	—	—	3.4	3.4	18.3	19.3	60.9	60.9	1.8	1.8	12.0	36.2	156.0	16.5	10.7	23.0					
Glycine	—	—	—	—	—	5.2	5.2	4.5	40.9	41.2	41.2	2.3	2.3	3.2	6.7	17.2	4.0	12.3	12.3					
Histidine	0.6	1.2	—	6.2	31.8	2.1	2.1	2.0	3.7	61.7	61.7	1.4	1.4	1.7	3.6	103.0	0.9	1.6	161.2					
Isoleucine	1.0	1.8	—	3.0	3.0	2.1	2.1	1.5	2.7	2.9	2.9	0.8	0.8	0.8	1.7	1.6	2.8	0.9	1.2					
Leucine	1.9	5.5	—	2.6	4.2	1.9	1.9	1.8	3.5	3.6	3.6	1.6	1.6	1.7	2.3	2.6	4.3	1.6	1.6					
Lysine	3.5	5.6	—	9.2	9.2	4.3	4.3	4.2	4.4	5.6	5.6	2.8	2.8	2.8	5.5	9.6	2.1	1.7	2.5					
Methionine	0.9	1.7	—	2.2	1.8	0.5	0.5	0.5	2.2	1.9	1.9	0.4	0.4	—	0.4	—	1.0	1.1	1.0					
Phenylalanine	0.9	2.8	—	2.1	2.1	1.1	1.1	1.1	2.1	2.2	2.2	0.9	0.9	1.0	1.0	1.6	2.3	1.3	1.3					
Proline	2.7	7.2	—	7.1	7.4	3.1	3.1	3.1	6.8	6.7	6.7	2.3	2.3	2.0	3.2	8.5	3.5	3.4	4.0					
Threonine	4.3	8.0	—	14.5	14.0	1.5	1.5	1.6	3.8	4.3	4.3	1.4	1.4	1.6	3.9	4.6	3.0	2.5	2.8					
Tyrosine	1.8	3.1	—	4.3	3.8	0.9	0.9	0.6	2.3	2.2	2.2	0.7	0.7	0.5	0.8	1.1	1.7	1.5	1.6					
Valine	2.1	4.1	—	3.2	3.1	2.8	2.8	2.8	4.3	4.3	4.3	2.4	2.4	2.4	2.3	2.9	4.1	1.5	1.3					
Total							54.1			224.6				40.0		345.8	56.4		223.5					
Serine												2.1	2.1	2.1	5.4	6.1								
Cystine												<0.2	<0.2	0.9	0.4	0.1								
Tryptophane												<0.2	<0.2	—	<1.05	—								
Ornithine												0.2	0.2	0.4	0.4	0.5								

Table 66. The free amino acids in the liver and brain of 40 gram male rats raised away from the mother (After Denton, Williams and Elvehjem [66])

	Liver γ/g wet tissue	Brain γ/g wet tissue
Arginine	27.5	47.7
Histidine	23.6	13.2
Isoleucine	48.1	6.1
Leucine	60.9	11.9
Lysine	96.8	
Methionine	50.0	10.9
Phenyl alanine	30.6	8.9
Tryptophane	14.1	4.8
Valine	50.6	13.9

leucine, valine, arginine, lysine, alanine, threonine, glycine, and serine. There are larger spots of γ-amino-butyric acid, taurine, and aspartic acid. The largest spots are those of glutamic acid, and somewhat smaller ones are those of glutamine. After hepatectomy, the spot corresponding to glutamine disappears and new spots of phenylalanine and tyrosine appear. Similar results are found in muscle: the spot corresponding to glutamine becomes larger, and that of glutamic acid smaller. New spots of phenylalanine and tyrosine appear [67].

In the tissue cells, various pathways of metabolism are available to the free amino acids. They can be incorporated into the existing tissue proteins (including the protein hormones), or they may be synthesized into new tissue protein. When the needs of metabolism require, a few of them are capable of conversion into glucose and a portion of their energy can be stored as glycogen for a period of time. Finally, the amino acids may be broken down in such a way that the products may be directly utilized to give off energy (oxidative catabolism). Various amino acids are metabolized into hormones or other essential compounds.

Synthesis and Metabolism of Tissue Proteins

Formation and Exchange of Amino Acids

Analysis of total metabolism teaches that protein is deposited during growth and catabolized during starvation. In adult animals and man, the total protein content of the body remains constant when the individual is in a state of balanced nutrition: as much nitrogen is excreted as is taken in in the diet.

Experiments performed with amino acids labelled with N¹⁵ have produced two important conclusions [68]. Only a part of the ingested isotopic amino acid nitrogen was excreted, between one-third and one-half. Since the nitrogen balance remained constant, and the weight of the animals did not rise, a part of the administered N¹⁵ must have been replaced in the process of excretion by non-isotopic nitrogen derived from body protein. Somewhere between one-half and two-thirds of the ingested N¹⁵-labelled amino acid was therefore incorporated into the body protein. Only a small portion of this was found in the fraction of amino acids not bound to protein, but it was present not only in those amino acids in which it was present when ingested, but also in other amino acids. Thus, when nitrogen balance is constant, the amino acids

of body protein are steadily interchanged with those present in the diet, and the nitrogen passes from the labelled amino acids in the food into other amino acids.

The isotopic N^{15} was not fixed by all tissue proteins to the same extent; i. e., the tissue proteins showed different exchanges of their own amino acids against those in the diet. The proteins of the intestinal mucosa, other internal organs, and the serum were the most active. The muscle proteins were less active. Since, however, muscle proteins comprise the largest portion of the body mass, they took up the largest total amount of N^{15} . The following gives, in descending order, the extent of nitrogen incorporation in the tissues: intestinal mucosa, fibrin, liver, kidneys, plasma globulin, total plasma, plasma albumin, pancreas, lymph nodes, spleen.

When C^{14} -labelled glycine, leucine, or lysine were given intravenously to mice, they disappeared from the blood within 10 minutes and were rapidly incorporated into the proteins of the viscera (after 30 minutes, about 75%) [69]. Soon thereafter C^{14} -labelled CO_2 appeared in the expired air, with maximum excretion after 1 hour. Shortly after the appearance of labelled amino acids in the visceral proteins, they also appeared in the proteins of the serum. An equilibrium between visceral protein and serum protein was established after 30 minutes. Ten to twenty minutes after the injection, a measurable amount of radioactivity was present in the muscle and skeletal proteins of the sacrificed animal; after 1 hour, this was almost completely gone. The individual amino acids were incorporated into proteins at different rates, between 3 and 4 microequivalents per gram of protein per hour.

In other experiments with rats using C^{14} -labelled glycine it was possible to determine how many microequivalents of amino acid were incorporated in 15 min. and after 6 hours per gram of tissue protein. The following results were obtained: intestinal mucosa 0.99 and 12.2; bone marrow 0.66 and 7.1; liver 0.8 and 6.8; kidneys 0.49 and 6.4; plasma 0.16 and 5.9; spleen 0.16 and 4.8; lungs 0.49 and 4.1; muscle 0 and 0.5; brain 0 and 0.3 [70].

The fact that the N^{15} was found not only in the administered amino acids shows that amino nitrogen is interchanged between the individual amino acids. It is chiefly the two amino-dicarboxylic acids which are involved in this exchange. Glutamic acid reacts somewhat more rapidly than does aspartic acid. Nitrogen can also be exchanged with several non-essential amino acids. Leucine, histidine, and tyrosine can give up their amino nitrogen for the synthesis of other amino acids, and their keto-acids can accept amino nitrogen. Lysine can readily give its α -amino nitrogen to other amino acids, but the corresponding α -keto acid is not changed back to the amino acid. This keto acid is also incapable of replacing lysine in growth experiments.

Schoenheimer [71] showed the exchange of the amino nitrogen of leucine by means of experiments with tagged leucine in which the amino group contained heavy nitrogen and, at the same time, the carbon skeleton was labelled with deuterium. When such double-labelled leucine was fed to rats, the body protein was shown to contain leucine in which the ratio of deuterium to N^{15} was shifted, as compared to the ratio in the administered amino acid, in favor of deuterium. This is only possible if a portion of the heavy nitrogen is replaced by normal nitrogen.

There are two possible ways in which the labelled amino acid could be converted into the tissue protein. Either the entire protein molecule is completely broken down into its individual components and then resynthesized, in which case the labelled amino acid replaces the normal amino acid previously present in the protein; or the peptide chains on either side of the amino acids which are to be replaced open up, and the new labelled amino acids enter. Until this has taken place, both free ends of the peptide chains must be held together.

There is considerable evidence that the first of these possibilities is the one that actually takes place. This has especially been demonstrated for antibodies, which of course are proteins [72]. The same conclusion was drawn by Simpson, Farber, and Tayer as the result of experiments with ethionine, which inhibits the incorporation of S^3 -labelled methionine and C^{14} -labelled glycine into tissue protein. Since it is possible to prevent the incorporation of two similar amino acids by means of one, these authors believe it unlikely that only a single amino acid is liberated and replaced [73].

Labelled amino acids are not only incorporated into the tissue proteins of intact animals, but also into tissue slices and tissue homogenates in vitro [69, 74, 75]. This fact allows further study of the mechanism of this action. Homogenates of liver incorporate L-lysine by 2 methods into 2 cell fractions. The particle-free material contains an enzyme system for this process, which acts best at pH 6.2 and in the presence of calcium ions. Another enzyme system of similar activity occurs in fractions of nucleus and mitochondria. It acts best at pH 7.4, and is also active in the absence of calcium ions. The first fact shows that, at least in the mature cell, protein can be formed independently of the nucleus [76]. Thus, labelled amino acids were synthesized in homogenates from which the nuclear material had first been removed by centrifugation. The individual cell fractions also synthesize other amino acids in various ways; e. g., glycine, leucine, histidine. This synthesis requires oxygen.

A labelled amino acid which is incorporated into a tissue protein disappears more or less rapidly according to the speed with which it is replaced by normal amino acids. The resulting exponential curves determine the *half-life of a given protein*. Sprinson and Rittenberg administered N^{15} -glycine to rats and to human beings and determined the excretion of N^{15} as urea. They discovered an equation by means of which they could calculate the protein turnover and the magnitude of the nitrogen stores. They found that the total protein of the rat had a half-life of 17 days; the half-life of the proteins of liver, plasma, and the other internal organs was 6 to 7 days; the half-life of the other body proteins was 21 days [77]. According to their findings, the human being turns over half of his total protein in 80 days, and half of his liver- and plasma-proteins in 10 days. Half of the protein of the lungs, brain, bones, skin, and of most of the muscle protein is renewed in 158 days [78].

Borsook and Deasy, using these methods, calculated that, for the total protein of the rat, 19 microequivalents of total amino acid per gram of tissue protein are turned over in 1 hour; for visceral protein, this figure is 57 microequivalents. For human beings, the values for total protein are 4.1 and for visceral protein 33 microequivalents. A man has 156 grams of protein per kilogram of body weight; hence, $156 \times 4.1 = 650$ microequivalents per kilogram per hour are turned over. The formation of a peptide bond requires about 3.5 calories. The energy requirement of the 650 microequivalents, on the assumption that only one peptide bond is formed, is $3.5 \times 0.65 \times 10^{-3} = 2.3 \times 10^{-3}$ calories per hour of protein synthesis per kilogram body weight; this is about 1/300 of the basal metabolism, which is approximately 1 calorie per kilogram per hour. Even if two peptide linkages are formed per amino acid radical, the energy requirement for protein synthesis constitutes only a tiny portion of the basal metabolism.

We shall now briefly discuss hemoglobin and the plasma proteins.

The *hemoglobin* of the non-nucleated erythrocyte does not belong to the dynamic or physiologically labile proteins. It is synthesized with the red blood cells, is stable for the life of the red blood cell, and finally is destroyed when the red cell is destroyed.

The life of a red blood cell is about 109 to 130 days, as determined by various workers using modern methods (M and N agglutination [79], use of Rh factor [80]). This same duration of life was found for hemoglobin, and indeed both for its protein and pigment components. N^{15} leucine is incorporated only into the protein components [81]. From its incorporation into globin, the life span of hemoglobin can be calculated to be approximately 120 days.

N^{15} glycine is also incorporated into the protein components; but it is necessary chiefly for porphyrin synthesis, where it supplies the nitrogen and a portion of the carbon [82, 83, 84]. (The precursors of protoporphyrin are formed chiefly in the liver [85] and in the bone marrow.)

The average life of the hemoglobin of the normal man was found to be 109 days; that of the normal woman, 130 days. The male therefore synthesizes 0.1 g of hemoglobin daily per kilogram of body weight; the female, 0.087 grams. These values correspond respectively to 3.45 and 3.00×10^9 erythrocytes per kg per day. In polycythemia, some 7.5×10^9 cells per kg are formed daily. The life span of sickle cells is only 40 days, and their formation is increased to 9.96×10^9 per kg per day, corresponding to 0.28 g hemoglobin. The red cells in pernicious anemia live for 72 days, and the corresponding daily production of new cells is 1.67×10^9 cells, or 0.0786 grams of hemoglobin per kilogram per day [86, 87]. The hemoglobin of sickle cells differs in various respects from normal hemoglobin [88].

It has been mentioned that labelled amino acids quickly appear in the protein of the liver and the blood plasma; for plasma this is probably due to the fact that plasma protein is synthesized largely in the liver. The experiments of Sprinson and Rittenberg (p. 347) have shown that a 70-kg. man daily synthesizes liver and plasma proteins corresponding to 6.2 grams of nitrogen; i. e., somewhat less than half of the total protein synthesis of the body, which corresponds to 15.3 grams of nitrogen.

Recently, W. Maurer and his coworkers fed rats S^{35} -methionine and followed the rise and fall of S^{35} in the proteins. They showed that the individual *protein fractions of the plasma* are synthesized at different rates. There is a daily replacement of 15 to 20% of the total serum protein by new protein. Half of the albumin is broken down in 5 days. The globulin fractions apparently are catabolized even more rapidly. The total protein content did not change during these experiments; hence, the rate of formation must be constant [90, 91].

In rats, 15 minutes after the administration of S^{35} -methionine the S^{35} appeared in the albumin fraction, demonstrating new synthesis of albumin. The S^{35} could be recognized in the α_2 -globulins after 30 minutes. The β - and γ -globulins were turned over more slowly, but then reached a higher rate of turnover. The same general results obtained when 30% of the total blood was previously removed by venesection [92]. The same results were reached by Oeffl. who injected I^{131} -labelled serum protein fractions into rats and rabbits. The albumins and the γ -globulins were more rapidly exchanged with extravascular protein than the α - and β -globulins [93].

In the larger animals and in man, synthesis and degradation go on more slowly. The half life of the serum proteins of rabbits is 10 days and that of human beings is about 30 days [94, 95, 96]. Prothrombin is synthesized with especial rapidity. When Dicumarol is used to prevent its synthesis in the liver, the prothrombin in the circulating blood disappears within 48 hours [97]. It must therefore be newly synthesized every 2 days.

Since the serum proteins are produced and turned over at various rates of speed, it must be concluded that their syntheses occur through different mechanisms and that, perhaps, they are synthesized at different locations in the liver cells [98, 99].

Table 67. The proteins of human plasma and some of their chemical properties and functions

Protein Fraction	% of the plasma proteins	Sedimentation Constant S _{20,w}	Isoelectric Point pH	Function
Albumin-globulinoprotein	0.5	3.5	3.0	Transport of copper
Ceruloplasmin	—	—	4.0	
Cholinesterase	0.005	—	4.5	
α_2 -globulin-globin	0.05	—	4.7	
Serum albumins	52.0	4.6	4.9	{ Binding of fatty acids, bile salts, drugs, mercury, pigments
Macroglobulin	(34)	—	—	
α_1 -globuloproteins	1.2	9	4.9	
α_2 -microproteins	0.5	9	4.9	
Fibrinogen	4.0	9	<5.3	Contain carbohydrates
Cold-soluble globulin	0.15	—	<5.3	Precipitated by barium
Antihemophilic globulin	—	—	—	Converted to fibrin by thrombin
α_1 -lipoproteins	3.0	5.0	5.2	{ Transport of steroids and carotinoids
α_2 -lipoproteins	5.0	7.0	5.4	
β_1 -lipid-poor euglobulin	{ 2.0 1.0 }	{ 7.0 20.0 }	5.5	
β_2 -metal-binding protein	3.0	5.0	5.8	
Isoagglutinins	(0.03)	—	6.3	Transport of iron and copper
β_1 -globulins	3.0	7.0	6.3	Agglutinate incompatible red cells
γ -globulins	11.0	{ 7.0 10.0 }	{ 6.3 7.3 }	
Accelerator globulin	—	—	—	
Prothrombin	0.3	—	—	
Heparin complement	—	—	—	Conversion of prothrombin
Plasminogen	—	—	—	Converted to thrombin by calcium and thromboplastin
Plasmin inhibitor	—	—	—	Reacts with heparin
Hypertensinogen	—	—	—	Streptokinase
Iodinated proteins	—	—	—	Plasmin
Complement components	{ C ₁ C ₂ } 0.4	—	—	Renin
Amylase	—	—	—	Formation of antigen-antibody complexes
Alkaline phosphatase	—	—	—	Splitting of starch
Peptidase	—	—	—	Splitting of phosphoric acid esters
β -glucuronidase	—	—	—	Splitting of 1-leucyl-glycyl-glycine
α_2 -protein	0.1	2.9	—	Splitting of glucuronides
β_2 -protein	0.05	5.0	—	Precipitated by barium

The experiments of Whipple et al. [98] suggest that the tissues synthesize their particular proteins from the proteins of the serum, without the necessity for the serum proteins to be broken down to amino acids before they pass into the tissue cells. When dogs whose protein stores were completely depleted by plasmapheresis received intravenous injections of plasma protein, they synthesized some 70 grams of tissue protein per day. This rate of synthesis was so rapid that the injected protein could not first have been broken down completely before it entered the cells. Bennhold and Seibold agree with this thesis [100].

The same results were obtained in experiments with antigens labelled with radioiodide [101] or fluorescent pigments [102]. The I^{131} -antigens were found in rabbit liver cells very shortly after their injection, both in the nuclear and the mitochondrial fractions. Antigens labelled with the pigments also appeared rapidly in the cells of the reticuloendothelial system and the renal tubules. Fischer has further shown that protamine and histone are taken up as such in the cells and can be demonstrated in them [103]. It is not yet established whether insulin and the other protein hormones act within the cells or at their surface (p. 212). If the first possibility is true, they must enter the cells unaltered. We do not yet know the process by which the plasma proteins are converted into the tissue proteins. Since the proteins of the blood plasma, as already discussed, probably play a role in the synthesis of tissue proteins, a table is reproduced from the work of Cohn et al. [111], which details the protein fractions of human plasma as far as they have been isolated and characterized to date (Table 67).

The *globulins* include a number of different proteins such as enzymes and their precursors, antibodies, certain factors necessary for the coagulation of blood, and others which can bind carbohydrate, lipids, metals, and other substances.

The *albumins* constitute the major portion of the plasma proteins (50–70%). They have not yet been completely separated into distinct fractions. One portion contains free sulfhydryl groups; another contains none. To date, it is their physical properties which are best understood. They maintain the suspension of the blood cells and the colloid stability of the plasma. In addition, they are involved in the transport function of plasma. They are especially concerned with the transport of small molecular compounds with polar groups, such as acid and basic dyes, inorganic ions, fatty acids, quinone derivatives, and various drugs including antibiotics. In addition, they maintain the colloid osmotic pressure and regulate the exchange of water between blood and tissues.

The extent to which the plasma proteins are involved in the synthesis of tissue proteins can be measured in the mammary glands during lactation. When lactating rabbits receive injections of α - C^{14} -glycine and other labelled amino acids, the milk proteins show a much higher activity six hours after injection than do the plasma proteins. The reverse would be expected if the milk proteins were synthesized exclusively from the plasma proteins. In the main, they are probably synthesized from free amino acids. It is not, however, excluded that a few products of catabolism of the plasma proteins may be used in the process [104].

Mechanism of Synthesis of Proteins and Peptides

In the *in vitro* and *in vivo* experiments cited above, individual amino acids of nutrient fluid or of the blood were incorporated into protein, and the identical amino acids were isolated from the resulting protein. These events serve to maintain body equilibrium. There are other situations, however, in which equilibrium is not maintained; such as, for example, those which occur during growth or in association with marked loss of protein (plasmapheresis, wasting diseases).

Feeding experiments have shown that growth can be maintained only when all the essential amino acids are supplied at the same time or at least within a period of a few hours of each other [104–108]. This is also true for the non-essential amino acids; either they must themselves be present, or the material from which they are synthesized must be present. The same is true for the synthesis of adaptive enzymes in yeast: when phenylalanine is lacking, maltase cannot be synthesized [109].

However, certain experiments in rats on a diet lacking an essential amino acid, gave conclusions which did not agree with this view. When tryptophane was absent, C^{14} tryptophane was incorporated more slowly than in the normally fed controls. However, when phenylalanine was lacking, C^{14} tryptophane and C^{14} lysine were incorporated at a rate which was only slightly less than when phenylalanine was present [110]. The previous feeding experiments would have suggested that both amino acids would not have been incorporated in the absence of an essential amino acid.

The *closing of the peptide bond* in the synthesis of peptides and proteins cannot be a simple reversal of hydrolysis. This fact is shown by energy studies, which show that peptide bond closure consumes energy. Each peptide bond of a chain does not have the same amount of energy. When a protein such as β -lactoglobulin is digested by pepsin, a process in which only peptides are formed, only 1300 calories are liberated per mol of split bond. The formation of a single dipeptide from two amino acids, on the other hand, requires 2,500–4,000 calories. Thus, at the beginning of the synthesis of a protein from a store of free amino acids in the tissues, a relatively large amount of energy must be supplied in the synthesis of the small peptides. Later, as the length of the chains increases, less and less energy is required [112].

Synthesis of Simple Peptides

The naturally-occurring systems for the study of these syntheses were the synthesis of hippuric acid and p-aminohippuric acid from benzoic acid or p-aminobenzoic acid, respectively, plus glycine; and the syntheses of glutathione and glutamine.

Under anaerobic conditions, or when respiration is reduced, neither hippuric acid [113] nor p-aminohippuric acid is synthesized [114]. For both syntheses, both adenosine-triphosphoric acid (ATP) and coenzyme A are necessary [115, 116]. ATP is formed by an oxidative process (p. 88). For this reason, the synthesis does not occur if oxidative phosphorylation is prevented; so it is clear that not only does 2,4-dinitrophenol prevent oxidative phosphorylation, but it also inhibits the synthesis of peptides and proteins, as well as the incorporation of amino acids into proteins. ATP is necessary for the activation of the carboxyl groups of benzoic acid [112]; it does not, however, react directly with benzoic acid, but first with an enzyme, E, not yet isolated, with the splitting off of inorganic pyrophosphate ($P \sim P$); the remaining adenylic acid (AMP) unites with the enzyme:



The complex E-AMP now reacts with coenzyme A (HSCoA) with the liberation of AMP and the formation of a new complex of the hypothetical enzyme E and coenzyme A:



In a third reaction, the benzoyl radical now replaces the enzyme in Coenzyme A:



That a benzoyl-coenzyme A compound is actually formed was shown by Schlächter and Taggart [118]. The enzyme which transfers the benzoyl group into coenzyme A is perhaps identical with the enzyme which activates the fatty acids. The other

enzyme, which then causes transfer of the benzoyl group to glycine, seems to be specific for this function (i. e., a "glycinacylase"). The synthesis of pantohippuric acid goes on in analogous fashion.

Of the two bonds in glutathione, the one between cysteine and glycine is a true peptide bond, while the one between the γ -carboxyl of glutamic acid and cysteine is more analogous to the acid amide bond of glutamine.

The synthesis of glutathione was elucidated by K. Bloch and coworkers in tissue cultures of pigeon muscle and pigeon liver [119-125]. This synthesis is also dependent on the presence of oxygen. A process of oxidation must thus again supply the energy for the synthesis of ATP. ATP is necessary for the formation of each of the two bonds of the tripeptide. Adenosine diphosphoric acid (ADP) inhibits this synthesis. Coenzyme A or some other coenzyme is not essential. No phosphorylated intermediate products have been isolated.

In the synthesis of the two peptide bonds, an enzyme first reacts with ATP, forming an enzyme-phosphate compound with the liberation of ADP [126, 127]:



The enzyme-phosphate then reacts with glutamic acid (Glu), with the exchange of the phosphate group for the carbonyl group of the glutamic acid:



From the glutamic acid-enzyme complex, the glutamic acid is finally transferred to cysteine (Cys):

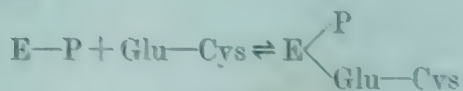


Purified "glutaminsynthase" (see below) catalyzes the synthesis of γ -glutaminylcysteine.

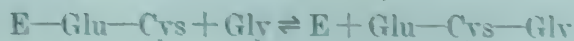
In the synthesis of the second, true peptide bond, enzyme-phosphate reacts with dipeptide γ -glutamyl-cysteine. The dipeptide either takes the place of the phosphate, or it is bound to the enzyme together with the phosphate:



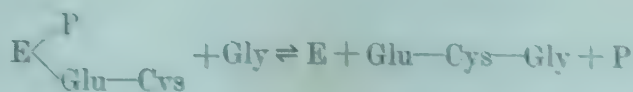
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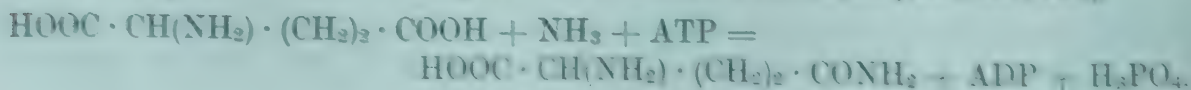
From either enzyme compound, the dipeptide is then combined directly with glycine [128, 129]:



or



As discussed above, the first peptide bond in glutathione is truly an acid-amide bond as in glutamine. For this synthesis too, ATP is essential [130, 131, 132]:



No intermediate phosphorylated products have been isolated in this reaction; here also it must be assumed that ATP first reacts with the enzyme to form enzyme-phosphate, and then the phosphate rest is exchanged with the glutamic acid rest and converted to ammonia.

In addition, this glutamine-synthetase can act as transferase, transferring the glutamic acid radical to, for example, ammonia or, as above, to cysteine.

When proteins are synthesized from free amino acids, the carboxyl groups are activated [137, 137 a, 137 b], but the mechanism of this process differs from that of peptide synthesis above. First an energy-rich mixed acid anhydride of an amino acid and adenylic acid is formed [137 c]. Extracts of animal and plant tissues as well as of microorganisms contain enzymes which catalyze this reaction [137 d]. There may be specific enzymes for the various amino acids [137 e, f]. At present, a tryptophane-activating enzyme has been isolated from beef pancreas [137 e, f], and methionine-activating [137 g] and tyrosine-activating [137 h, i] enzymes from yeast. Hog pancreas has given enzymes which activate tryptophane, tyrosine, threonine and serine [137 k, l]. Only about half of the naturally occurring amino acids have been converted into the mixed anhydrides [137 a], but it is likely that the others are activated in a similar way [137].

These enzymes require ATP for the activation process. Inorganic pyrophosphate is split off and replaced by the amino acyl residue [137 m, n, o]. The amino acyl AMP-anhydride formed remains bound to the enzyme. Free amino acyl-AMP-anhydride is not stable.

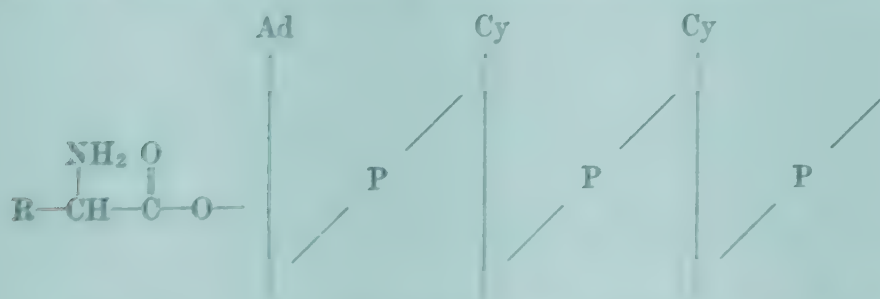
Magnesium ions must be present in this reaction.



The reaction is reversible. The enzymes are found in the cytoplasm.

The activated amino acids yield hydroxamates with hydroxylamine and can be determined colorimetrically [137 o]. The amino acid-AMP-anhydrides react readily with free amino acids to form peptides [137 p, q].

If one uses labelled amino acids in these experiments, they can be recovered in the nucleoproteins of the microsomes. The labelled amino acids do not pass here directly but by way of an intermediate product which is a polynucleotide dissolved in the cytoplasm. Its molecular weight is approximately 20,000 to 35,000 [137 r, s]. The activated amino acid is bound to the 2' or 3' hydroxy group of a terminal adenosine nucleotide [137 t, u] which is preceded by two cytidylic acid residues.

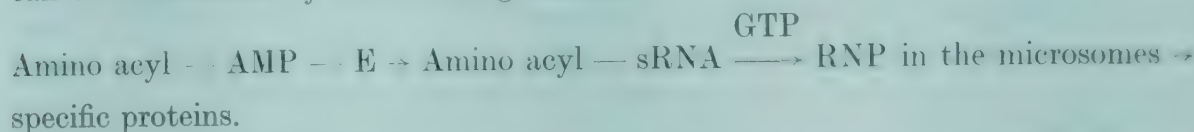


According to this scheme, one molecule of soluble acceptor-RNA binds only one amino acid. Several acceptor-RNA's may exist which are specific for the individual amino acids [137 t, u]. However, Koningsberger et al. [137 v] have isolated from yeast an s-RNA to which a peptide was bound. If this is also true for other tissues, one

could conclude that several complexes of amino acyl s-RNA transfer their amino acid residues one after the other to a definite amino acyl s-RNA to form a peptidyl s-RNA complex.

Finally, the amino acyl residue and ultimately also the peptidyl residue are incorporated into the nucleoproteins of the microsomes [137 w z]. Perhaps polypeptides are formed here as intermediate products. Guanosine-triphosphate (GTP) must be present for this reaction [137 y]. We do not yet know the pathway from such a nucleoprotein to a specific protein.

The steps from the mixed acid anhydride, bound to the specific enzyme, to protein can be summarized by the following schema:



In addition to the cytoplasm, the nucleus is also able to synthesize proteins from amino acids. This has been demonstrated in experiments with isolated nuclei of thymus cells by V. G. Allfrey et al. [137 aa]. In this case, the DNA controls the protein synthesis, because synthesis does not take place when DNA has been removed by DNase, and starts again when DNA of thymus or other organs is added. Even RNA can re-start synthesis. Isolated nuclei from rat liver behave similarly [137 ab]. It is not known if the DNA controls the protein synthesis directly or by way of RNA as a mediator. However, in the first stages of the developing egg, the protein synthesis seems to be governed by the DNA alone [137 ac].

Observations on patients, which will be discussed in the next chapter, have demonstrated that the presence as well as the absence of specific proteins and enzymes are genetically controlled, but it is not known how the information is transmitted from the gene to the protein. A gene is probably a desoxyribonucleoprotein, a compound of a protein with DNA. In the somatic cells, this protein is a histone. The nature of the nucleoproteins in the nuclei of the gametes can only be inferred from investigations of the nuclei of mature fish sperm. Here the protein is nucleoprotamine, a salt of the basic protamine and DNA. In this case, a gene must be a nucleoprotamine. The composition of the protamine varies from one species of fish to another and is quite characteristic. On the other hand the analysis of the corresponding nucleic acids has shown no significant differences [137 ad]. Nevertheless, most authors believe that the DNA contains all the information on heredity and expect that the future knowledge of the sequence of the nucleotides in the DNA will reveal the secret.

The information in the DNA will be communicated to the RNA, which will transmit it to the ribonucleoprotein and from here to the protein itself.

The role played by ATP in the *synthesis of proteins* also explains the well-known fact that carbohydrate spares protein. The most important function of carbohydrate metabolism in this regard is the production of ATP. This can be utilized for synthesis of proteins only if the carbohydrate enters the metabolic process at the same time as, or within a short time after, the amino acids derived from the proteins of the diet [138, 139, 140].

As carbohydrate is taken up, more ATP is produced; at the same time, the free amino acids in the blood and tissue rise, and more of them can be activated.

Borsook observed that rabbits given both amino acids and additional glucose showed an increase in reticulocytes, denoting the production of more protein, especially hemoglobin [132].

Botssok also explained the *specific dynamic action* of proteins or amino acids on the basis of the formation of ATP [132]. A short time after a protein meal, the metabolic rate goes up and later falls to the initial value. There are parallel rises and falls of the amino acid levels in the blood and, probably, the tissues [141]. At the same time, more ATP is used up and more carbohydrate must be catabolized to cover the increased need for ATP. As a result, the specific dynamic action may correspond to the increased energy requirement for protein synthesis.

From Peptide to Protein

If peptide is to be converted to protein, not only must the necessary amino acids be joined together, but the joining must be done in the correct order. The mechanism which assures that the correct order will always be reproduced is quite obscure. Various factors must work together in this regard. In no living organism is protein built from one isolated collection of free amino acids, but only in the presence of another protein; the conclusion is therefore reasonable that the preexisting protein serves as model for the protein to be newly built [142].

The number of possible ways in which the amino acids may be arranged is astronomical. For a theoretical protein containing 20 different amino acids, and only 1 molecule of each, the number of theoretical arrangements is 2.4×10^8 . In actual fact, however, the proteins do not always contain all 20 amino acids, but rather a varying number of molecules of particular ones. This fact increases the number of possibilities to an unmeasurable amount. Without further discussion, it can be seen that each tissue can have its particular protein, and that it can vary from individual to individual and from one animal to another. Hypersensitivity reactions allow us to investigate the multiplicity of the proteins. An individual sensitized against the globulin of horse serum does not react with the globulin of sheep serum. The actual events never reach the theoretical possibilities. There must be some basic orderly principle which chooses only certain of the innumerable possible arrangements of amino acids, and maintains the proper individual arrangement in individuals of successive generations. Many investigations of the last 10 years have confirmed that nucleic acids are concerned with protein synthesis [143, 144]. Their metabolism increases markedly when more protein is synthesized. Above all, ribose nucleic acid is concerned with the daily synthesis of proteins. Desoxyribosenucleic acid is much less metabolized and is probably concerned only with the formation of chromosomal protein, for it is found exclusively in the cell nucleus, while ribose nucleic acid is found both in the cytoplasm and in the nucleus. The maximum synthesis of protein coincides with the metabolism of nucleic acid in the regenerating liver, or follows it closely [145, 146].

Sometimes, however, the two processes may go on independently of each other. Thus, under certain conditions, 140 cc of rabbit reticulocytes form 300 mg of hemoglobin in 4 hours, while at the same time the concentration of ribose nucleic acid falls [147]. Experiments with bacteria have shown that certain specific substances inhibit the incorporation of labelled glycine into protein more than into the purine bases of the nucleic acids [148].

There are various theories as to the manner in which the nucleic acids may operate. Haurowitz [149] suggested one such theory. When new protein is to be synthesized on the model of a preexisting protein from amino acids, these must first be arranged in the same order as they are found in the model protein. Crystalline forces can attract the same amino acids, but only over short distances. Because the peptide chains in

proteins are folded, the attraction at the inner portions of the molecule cannot operate on the free amino acids. One property of the nucleic acids is to bind the basic groups of the peptide to their phosphoric acid radicals and thus to cause unfolding of the chains, so that all the components are freely accessible. The free amino acids can now be absorbed in the proper order and finally can be joined together to form the peptide chain. As a final step, the new peptide separates itself from the model peptide and becomes folded (Fig. 70).

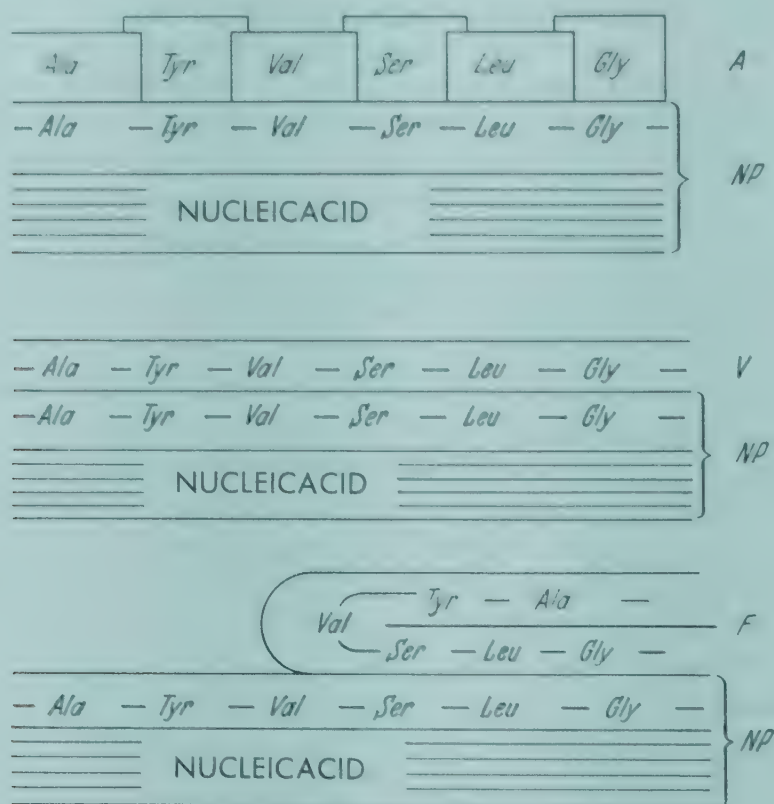


Fig. 70. The synthesis of a peptide chain according to a model protein.

A: adsorbed amino acids, NP: nucleoprotein model, V: coupling of the amino acids to form a peptide chain, F: folding of the peptide chain; Ala: alanine, Tyr: tyrosine, Val: valine, Ser: serine, Leu: leucine, Gly: glycine.

Lipmann postulates that there are specific sites in the enzymes concerned with protein synthesis, which activate special amino acids and join them to each other. The arrangement would then be predetermined by the enzyme. Perhaps the model protein itself is incorporated into the enzyme [117].

On the basis of experiments on bacteria, Gale attributed a very specific function to nucleic acid. The rate of growth of bacteria is very rapid, and because synthesis of protein and nucleic acid is of importance in growth, bacteria present a suitable medium for the study of the relationships between protein synthesis and nucleic acid synthesis. In *staphylococcus aureus*, the content of nucleic acid can be reduced by means of antibiotics; at the same time, the synthesis of protein is markedly reduced [150]. The dependence of protein synthesis on nucleic acid becomes even more clearly apparent in experiments in which the cells are broken up by supersonic waves. The crushed cells still synthesize protein, but synthesis stops when ribose nucleic acid is taken away and starts again when ribose nucleic acid or desoxyribosenucleic acid is

supplied. The nucleic acids govern not only the synthesis of protein, but also the incorporation of individual amino acids and the experimental exchange of C^{14} -labelled amino acids for the normal amino acids present in the protein.

In such experiments with *bacterial fragments*, the ribose nucleic acid of staphylococci could not be replaced by ribose nucleic acid from yeast or from other plant and animal tissues. This fact argues for specificity of synthesis. If the nucleic acid is removed little by little rather than all at once, the incorporation of all amino acids is not inhibited at the same time; that of glycine, aspartic acid, and leucine is inhibited more rapidly than that of alanine and lysine. Thus, certain of the nucleic acids must be specifically involved with certain definite amino acids, and must be released at varying rates of speed.

Yeast nucleic acid, which as a whole cannot replace the nucleic acid of bacteria, must also contain portions which are specific for the same amino acids. For example, when it is broken down by ribonuclease to polynucleotides, it can replace the nucleic acid of staphylococcus with respect to the incorporation of certain amino acids. In the original yeast nucleic acid, the determinative structures are arranged in the characteristic form of yeast protein. After they are broken down by the enzyme, they can enter individually into chemical actions. Protein and nucleic acid must thus harmonize with each other.

From the mixture of polynucleotides which ribonuclease produces from staphylococcal nucleic acid, Gale and Folkes were able to isolate, by means of electrophoresis and chromatography, several fractions which encouraged the incorporation of quite specific amino acids. One such fraction, that which governs the incorporation of aspartic acid, is probably an adenine-cytosine-dinucleotide. Another fraction, which is specific for glutamic acid, is probably a trinucleotide with one guanine and 2 uracil residues [151, 152, 153].

Before one can make the general conclusion from these studies that the arrangement of the amino acids in a protein to be synthesized preexists in the structure of the nucleic acids, one must await the results of studies with other tissues.

When a given order is present, the new question arises as to how the corresponding arrangement of the structures in the nucleic acid is produced. This order is probably controlled by the protein.

Transformations of Proteins

In accordance with this presentation, it might be concluded that the protein necessary for growth and repair is synthesized only from the store of free amino acids. This is usually the case, as in the growth of bacteria, the synthesis of adaptive enzymes, and the synthesis of hemoglobin in the erythroblasts and in the reticulocytes.

In some cases, as in the formation of antibodies, the new protein is produced so rapidly that synthesis from amino acids is not possible. In all probability, in such cases, preexisting protein in the cell is converted into a new protein, so that peptides or amino acids are split off or newly produced; or, both processes may occur at the same time. There are enzymes known as "transpeptidases" which convert glutamic acid radicals to other amino acids and perhaps even to peptides [154-157], but it is not known if similar enzymes exist for other peptides and amino acids.

There are many other examples of the formation of proteins from other proteins, such as the activation of pepsinogen and trypsinogen [41], the transformation of

fibrinogen into fibrin [158, 159], etc. In all probability, the protein hormones of the hypophysis are synthesized in this way from one or several "mother proteins," for this organ is especially rich in proteolytic enzymes. A recent example is supplied by Roka, who demonstrated, in liver homogenates, the conversion of factor VII, a plasma protein, into prothrombin [160].

The oldest and best known example of a transformation of proteins is the *synthesis of protamine* in the maturation of fish spermatozoa. In the testes of bony fish, one can detect protamine in the cell nuclei only from the time when spermatozoa can be recognized [161, 162]. During the time of maturation, the fish do not ingest any food, so that the protamine must be formed from other preexisting protein, probably from the protein of the trunk muscles. The chief properties of protamine are its simple structure and its basic nature. Protamines consist of only a few amino acids, in which the basic, especially arginine, predominate. There are no sulfur-containing amino acids and, with few exceptions, no aromatic amino acids. The protamines are formed from complicated proteins through a series of simplifications until a single radical is left behind which serves to help transmit hereditary characteristics and to bind desoxyribosenucleic acid. The amino acid model of protamine varies from one species of fish to another, and is characteristic for each. The protamines are bound to nucleic acids as salts.

At first, the occurrence of the protamines seemed to be limited to the bony fish. In recent years, however, they have also been found in the spermatozoa of the rooster. They may also be found in the spermatozoa of the higher mammals, for these are rich in arginine. Thus the protamines and the events which lead to the formation of the protamines acquire general importance.

During the development of the fertilized egg, complicated proteins must once again be synthesized from protamines, so that all the other amino acids must again be supplied. These are probably derived from the yolk of the egg. If the above view concerning the function of the nucleic acids in protein synthesis is confirmed, one can assume that the model of the subsequent series of amino acids which are to be formed preexists in the desoxyribosenucleic acid of the nucleus of the spermatozoa.

The position of the polypeptides in protein metabolism [162 a].

Polypeptides can originate in metabolism at various sites. First they can be formed during the biosynthesis of proteins because the hundreds of amino acids occurring in a protein perhaps do not unite at the same time but in steps, first building shorter and later longer chains. However, according to the authors who have investigated this problem experimentally, the synthesis of proteins does not begin until all constituting amino acids are assembled [162 b]. If one essential amino acid is missing it is unlikely that the synthesis proceeds up to the point where this amino acid enters and then continues after it has arrived. Under such circumstances the synthesis does not begin at all [162 b].

The mammary gland synthesizes the milk proteins, especially the casein and β -lactoglobulin from free amino acids of the blood. If C^{14} -labelled lysine is given to lactating goats, it is equally distributed over the peptide chains of casein [162 c]. This is only possible if it enters into its several sites at the same time. If the synthesis proceeded stepwise, the distribution would be unequal. Therefore one currently assumes

that all amino acids of a protein to be synthesized are combined at the same time. If the serum proteins are to be used for the synthesis of the milk proteins, hydrolysis must first take place.

If bacteria are used for the adaptive formation of β -galactosidase they can synthesize the enzyme only if all necessary amino acids are available [162 d]. On the other hand, slices of mouse pancreas form the proteolytic enzymes faster when the mixture of peptides produced by peptic digestion by chymotrypsin is added to the suspension fluid [162 e].

The synthesis of some proteins may proceed in a different manner. Glycine seems to be incorporated in muscle collagen with varying velocities at different sites in the molecule [162 f].

The exact significance of the peptides in protein synthesis is thus not known at this time. It seems unlikely that they are merely a transition step in protein synthesis, but a small fraction of the peptides found in the extract of an organ may be the result of interruption of synthesis.

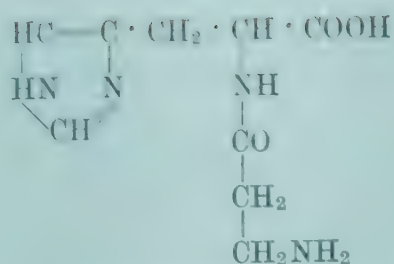
Most tissue peptides are probably formed by the decomposition of proteins and these peptides may exist for a time according to the specificity of the involved enzymes and the conditions of their activity. All tissues contain proteolytic enzymes which cleave peptide bonds between definite amino acids and decompose the proteins into fragments of varying lengths. These can exist until the polypeptidases, which are also present in all tissues, begin to act.

The specificity of the tissue proteinases is not yet known. Some of them act like pepsin; others, like trypsin or chymotrypsin.

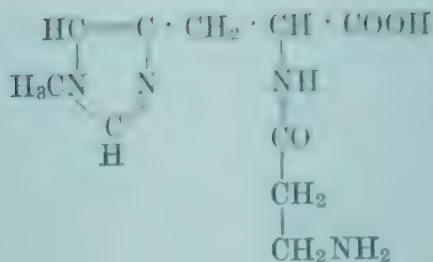
Proteolytic enzymes are also present within the blood. Thrombin is a proteinase; plasmin is a second proteinase, which dissolves fibrin. Plasminogen, its precursor, is found among the euglobulins and can be activated by several enzymes such as streptokinase, tissue proteinases, and urokinase [162 g]. The active plasmin also hydrolyzes other proteins besides fibrin and can produce active peptides from plasma proteins if its concentration is sufficiently high [162 h, i, k, l]. The presence of proteinases in the tissues becomes apparent in autolysis, when they decompose the proteins of their own cells. It is not known which proteins are the normal substrates in the living tissues. Proteinases may participate in the conversion of one protein into another, for it is likely that a cell produces only a few proteins from free amino acids and transforms them into specific ones according to the need of the moment by removing amino acids or peptides.

If an active enzyme is formed from its inactive precursor, the process is usually nothing else than a transformation of one protein into another. As a rule, amino acids or peptides are split off. This has been demonstrated for the activation of pepsinogen, trypsinogen, chymotrypsinogen and prothrombin to the respective enzymes. The conversions of fibrinogen to fibrin and of casein to paracasein are also in essence limited hydrolysis, and peptides are split off. Most peptides found in tissue extracts, blood, urine and other body fluids are produced in this way [162 m]. Normally they exist for only a short time and are soon completely decomposed. If they pass the kidney during their circulation in the blood, some may be excreted into the urine. Abnormal peptides occur in the blood and urine in disease states.

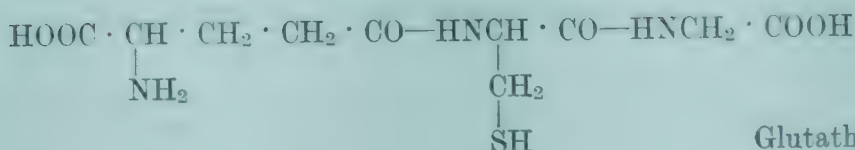
The peptides discussed are intermediate products of protein synthesis, degradation and transformation. In addition, many organs produce peptides with a definite function. The two dipeptides carnosine and anserine, and the tripeptide glutathione, are well known:



Carnosine



Anserine



Glutathione

Carnosine and anserine probably participate in the transfer of phosphates in the muscle. Glutathione is important in the transport of hydrogen in biological oxidation and in the transformation of methylglyoxal to lactic acid [162 n] and of formaldehyde to formic acid [162 o]. In the first reaction, S-lactyl-glutathione is an intermediary product.

The lens of the eye contains large amounts of glutathione and two similar tripeptides, ophthalmic and norophthalmic acid. In these two compounds, cysteine is replaced by another amino acid, α -aminobutyric acid in ophthalmic acid and alanine in norophthalmic acid. Their function is poorly known; ophthalmic acid is supposed to inhibit enzymatic synthesis of glutathione by lens extracts [162 p].

The proteohormones are among those peptides which have definite functions. The content and sequence of amino acids have been determined in several proteohormones by the following procedure. The amino acids are first determined quantitatively and then the end amino acids are determined by one of several methods. The Sanger method [162 q] determines the N-terminal amino acid by adding fluoro-2,4-dinitrobenzene in slightly alkaline solution to a solution of the peptide or protein under study. This substance reacts with the free terminal amino group (in the case of terminal proline or hydroxyproline, with the imino groups) and with the ϵ -amino group of lysine, yielding dinitrophenyl derivatives. If the dinitrophenyl peptide or protein is then hydrolyzed, the dinitrophenyl residue remains chiefly with the respective amino acid and the DNP-amino acid can be identified by paper chromatography. Another method has been described by Edman [162 r], who uses phenyl-isothiocyanate, which forms the phenylthiocarbamyl derivative of the peptide or the protein. The terminal amino acid can then be split off as thiohydantoin by hydrochloric acid. The amino acid is then liberated by alkaline hydrolysis and determined by paper chromatography.

The amino acid at the carboxylic end cannot be readily determined. Of several methods, the best is that which utilizes hydrazinolysis, reaction with N-bromosuccinimide and hydrolysis with carboxypolypeptidase. The hydrazinolysis [162 s] has been worked out by Akabori and coworkers. The peptides and proteins are heated with hydrazine. Those amino acids which are bound in peptide linkages are converted into hydrazides, but those at the carboxylic end are liberated as such.

N-bromosuccinimide splits off carbon dioxide from the terminal amino acid, and the corresponding amine can be isolated following hydrolysis [162 t].

25 26 27 28 29 30 31 32
 - ala · gly · glu · asp · asp · glu · ala · ser · -

α -corticotropin of sheep

The amino acids 1 to 24 are responsible for the action on the adrenals. The amino acids 25-39 are not essential and can be removed without influencing the activity.

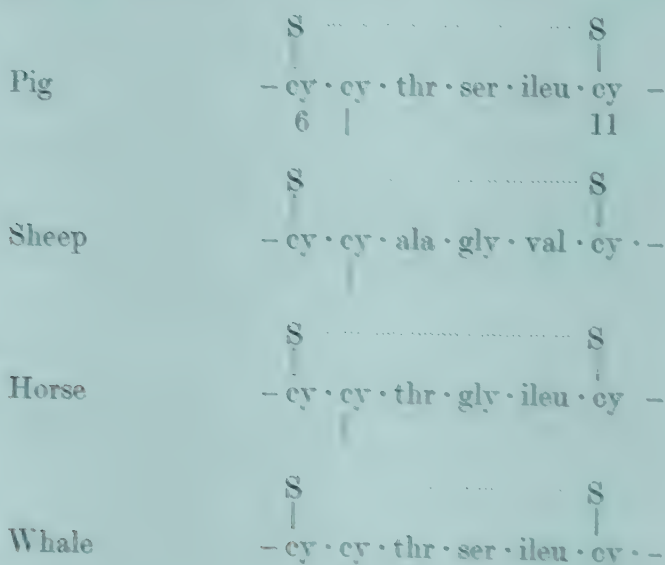
The pure corticotropins stimulate the melanocytes to expand. This action is due to the similar structure of these compounds to that of the real melanocyte-stimulating hormones, the intermediins. Two intermediins are known, α and β . The α -intermedin consists of the first 13 amino acids of β -corticotropin with the exception that the free amino group of serine is substituted [162 y]. The β -intermedin has the heptapeptide - met · glu · his · phe · arg · try · gly · - in common with the β -corticotropin and the α -intermedin. To the left of this heptapeptide there are six amino acids in β -intermedin and only three in α -intermedin and in β -corticotropin [162 z].

β -Corticotropin	ser · tyr · ser ·	met · glu · his · phe · arg · try · gly ·	lys · pro · val · gly · · ·
α -Intermedin	R · ser · tyr · ser ·	met · glu · his · phe · arg · try · gly ·	lys · pro · valNH ₂
β -Intermedin	$\left\{ \begin{array}{l} \text{asp · glu · glu ·} \\ \text{pro · tyr · lys ·} \end{array} \right\}$	met · glu · his · phe · arg · try · gly ·	ser · pro · pro · lys · asp

common heptapeptide

Insulin can be ranked within the peptides because its molecular weight is only 6000 according to recent experiments. It consists of two peptide chains which are linked together by two disulfide bridges. Chain A contains 21 and chain B 30 amino acids. In position 6 and 11 of chain A are two cysteine residues which are connected by another disulfide bridge [162 aa].

Insulin preparations isolated from other animals have a different structure but only between the 6th and the 11th amino acids of chain A as shown in the following formulas [162 bb].

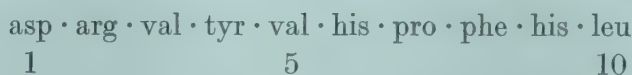


These slight differences seem to be genetically controlled.

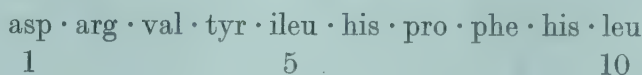
The extracts of many organs increase or decrease the blood pressure. The responsible substances (kallikrein, kallidin, substance P, substance U) are pharmacologically well characterized but not biochemically. Most of them are polypeptides because they are inactivated by proteolytic enzymes. It is not known if their action on blood circulation is of functional importance or if this activity is only an accidental quality. However they are regularly found in the respective organs [162 cc].

Apart from the peptides produced in normal metabolism and from the proteohormones there occasionally appear other peptides under physiological and especially under pathological conditions. Usually they are formed when enzymes act upon unusual substrates or when an enzyme at its normal site does not have enough time to break down its usual substrate. In both instances peptides may appear outside of the normal protein metabolism. These "wild peptides" are sometimes active pharmacologically and disturb the tissues and organism by displaying their activity at the wrong place and at the wrong time. It may be assumed that every protein contains active combinations of amino acids which can occasionally be liberated by enzymes.

The classical example of such a peptide is hypertensin, which is formed if renin, a proteinase of the kidney, enters the blood and acts on the α_2 -globulin. This happens when the permeability of the kidney cells is pathologically increased by disease [162 cc]. The reaction can be imitated in vitro by adding renin prepared from rabbit kidney, to beef or horse serum. Renin hydrolyzes the serum proteins to a mixture of peptides, one of which increases the blood pressure. This has been isolated and shown to be a decapeptide. That found in beef serum has the following structure [162 dd]:



The decapeptide from horse serum contains isoleucine instead of valine in position 5 [162 ee]:



A second active peptide has been prepared from horse serum after digestion with renin, which contains the amino acids 1-8 of the decapeptide. The two last amino acids, histidine and leucine, are therefore not necessary for the activity [162 ff]. Another octapeptide of the first 8 amino acids of beef hypertensin has been synthesized, which has the same effect. All these peptides have three to four times more effect on the blood pressure than norepinephrine.

It is likely that enzymes from other tissues can pass into the blood and lymph and liberate active peptides from proteins. Some authors, for example, claim that every damage to tissue accompanied by inflammation is accompanied by proteolysis [162 gg, hh]. It has been shown that active peptides are present in inflammatory exudates. On the other hand, the signs of inflammation can be produced by the products of trypsin and pepsin digestion of fibrin and gelatin and by crystalline peptides [162 hh, ii]. When tissues are damaged mechanically or by burns, enzymes pass from the cells and produce active peptides from foreign proteins [162 kk].

Degradation and Synthesis of the Amino Acids

There are several alternative routes of metabolism even for those free amino acids of the tissue which are not used for the synthesis of protein. In the most common pathway, they are broken down to carbon dioxide, water, and urea; the energy which they contain is made available for varying purposes.

In most amino acids, the process of degradation begins at the amino group. A few amino acids have, in addition to the amino and the carboxyl groups, a third reactive group at which the splitting process may sometimes begin; the amino group in such cases is then removed at a later stage.

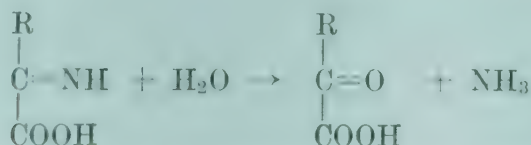
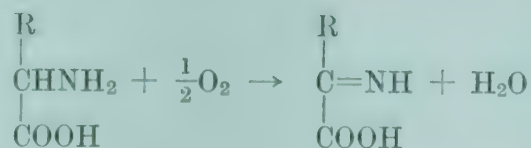
A few amino acids are decarboxylated, and specific "decarboxylases" have been found in various animal and plant tissues. The resulting amines can no longer be completely broken down; that is, utilized for energy production.

Finally, a few amino acids are converted into active materials. In this process, there is usually an associated decarboxylation.

Deamination

The first clue as to how the amino group may be removed was discovered by Otto Neubauer [163]. He fed dogs and men phenylaminoacetic acid and isolated phenylglyoxylic acid and benzoic acid from the urine. He concluded that the amino group was split off in the presence of oxygen, with the amino acid being converted to the α -keto acid; from this product, as a result of decarboxylation and oxidation, the next lower fatty acid was produced.

Knoop [164] analyzed such oxidative deamination in more detail and proposed the following equation:



Today, this equation is stated somewhat differently. Instead of half a molecule of oxygen, a whole molecule is involved in the reaction, and hydrogen peroxide, not water, results. As a rule, the hydrogen peroxide is converted by catalase into water and oxygen. If this did not occur, the hydrogen peroxide could immediately oxidize the α -keto acid to the next lower fatty acid.

The processes of oxidative deamination have been confirmed by many other authors, mostly with synthetic amino acids which are foreign to the body. Imino acids are assumed as intermediate products; none have yet been demonstrated, but their presence is inferred from indirect evidence. For deamination, hydrogen must be present at the α -carbon atom, and at most only one of the 2 hydrogens of the amino groups may be replaced by, for example, a methyl group.

Experiments with tissue slices and tissue broths have shown that, although the L-form of amino acids is generally more widespread in nature, it is the D-forms of the

amino acids which are more rapidly deaminated. Krebs [165, 165 a] has isolated a *D-aminoacidoxidase* from liver and kidney cultures of various animals. This enzyme requires, as a coenzyme, flavine-adenine-dinucleotide [166, 166 a, 167]; it deaminates almost all D-amino acids but not, for example, D-glutamic acid. The exact significance of D-aminoacidoxidase in metabolism is not yet clear; for the intact animal utilizes ingested D-amino acids much more poorly than L-amino acids [168]. A few D-amino acids are present in plant tissues, but play no quantitative role; they may be deaminated by this enzyme and their carbon skeleton then enters the normal metabolic processes.

Another flavine enzyme oxidizes glycine to glyoxylic acid and ammonia [169]. This enzyme has been demonstrated in various tissues, but it is not yet known to what extent it actually is active in intact tissues.

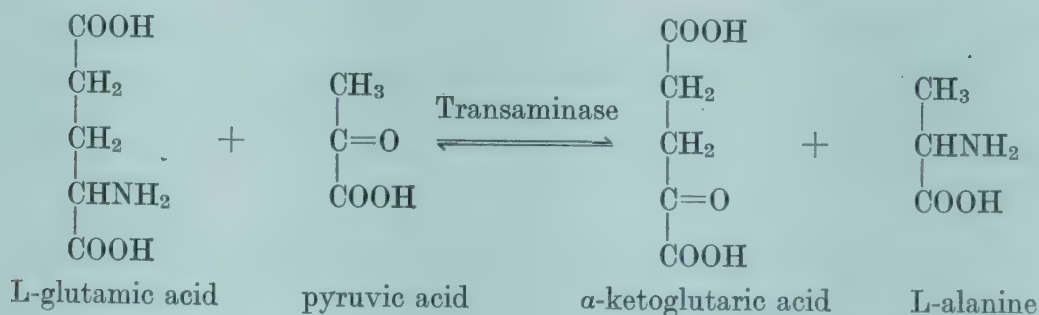
Finally, an *L-aminoacidoxidase* is also said to exist [170, 171, 172] which effects oxidative deamination of a number of L-amino acids. Such an L-aminoacidoxidase which was isolated by Blanchard et al. from rat kidneys requires riboflavinphosphoric acid as a coenzyme [173]. Its activity, however, is very small, and it is questionable whether it is actually involved in normal cellular metabolism. This L-aminoacidoxidase of rat kidneys does not act on aspartic acid, glutamic acid, serine, or threonine.

On the other hand, liver contains a dehydrogenase for L-glutamic acid which converts it into α -ketoglutaric acid [174].

It thus appears that only a few of the L-amino acids which are involved in metabolism give up their amino groups through oxidative deamination. If one adds L-amino acids to liver or kidney cultures, they do use oxygen and are converted as a rule into α -keto-acids, most of which are immediately further broken down. Seldom, however, is the expected amount of ammonia or urea produced. Therefore, their amino groups must be removed by means of another reaction: transamination.

Transamination

Transamination was discovered by Braunstein and Kritzmann [175, 176]. In transamination, an amino acid exchanges its amino group with the doubly-bound oxygen of an α -keto acid, so that the amino acid is converted into an α -keto acid, and the α -keto acid into an amino acid. Thus, for example, when L-glutamic acid reacts with pyruvic acid, α -ketoglutaric acid and alanine are formed:



At first, it was believed that only alanine, aspartic acid, and glutamic acid, as well as their corresponding keto-acids, could participate in this reaction [177]. Experiments with N^{15} -labelled amino acids, however, have shown that N^{15} occurs in other amino acids soon after it is administered [178]; it must therefore be assumed that virtually

all amino acids can be converted into keto acids by means of transamination. Conversely, it is possible to maintain the growth of animals and bacteria by means of the keto acids of the essential amino acids, if the amino acids themselves are lacking in the diet or the culture medium, respectively.

The chief keto acids into which the amino acids are converted are pyruvic acid, α -ketoglutaric acid, and oxalacetic acid.

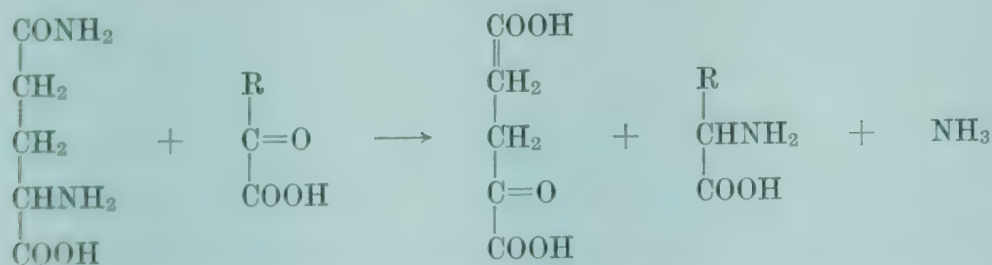
In vitro, α -ketoglutaric acid usually reacts more rapidly than the other 2 keto acids. In crude preparations of various animal tissues, the amino groups of the following amino acids can be transferred to α -ketoglutaric acid: glycine, alanine, serine, cysteine, α -aminobutyric acid, aspartic acid, taurine, methionine, ornithine, valine, leucine, isoleucine, norleucine, phenylalanine, tyrosine, dihydroxyphenylalanine, histidine, and lysine [179]. Cysteinic acid can also be converted in this manner into the corresponding α -keto acid [180].

The process of transamination is catalyzed by various transaminases, some of which are highly specific. None has as yet been isolated in pure form. However, the existence of special transaminases for the reactions glutamic acid \rightleftharpoons aspartic acid, glutamic acid \rightleftharpoons alanine has been demonstrated. In the reaction aspartic acid \rightleftharpoons alanine, glutamic acid is probably interposed in the sense that aspartic acid first reacts with α -ketoglutaric acid and then the resulting glutamic acid reacts with pyruvic acid [181, 182].

Tyrosine reacts in liver cultures directly with pyruvic acid [5].

It has recently also been shown that the amino group of an amino acid can be transferred to glutamic acid, and that this can be converted into glutamine. In kidney cultures, for example, the amino group of tyrosine is transferred to the γ -carboxyl group of glutamic acid [183]. Probably, aspartic acid can take up ammonia in like manner.

The amides of the two amino-dicarboxylic acids also play a large part in the amination of the α -keto acids [184]. There are 2 enzymes present in the liver which have to do with the splitting of the amido groups of glutamine. One deaminates only in the presence of phosphate; the other, only in the presence of an α -ketoacid [185, 186, 187, 188]. The same is probably true for asparagine. If a keto acid reacts with L-glutamine and this second enzyme, stoichiometric amounts of ammonia, amino acid, and α -ketoglutaric acid are formed. The ammonia comes exclusively from the amido group.



D-glutamine cannot substitute for L-glutamine in this reaction. If L-glutamine is replaced by L-glutamic acid, the transamination goes on at a much slower rate or does not go on at all.

Transamination precedes deamidation; an intermediate product is α -ketoglutaric acid amide or, in the case of asparagine, oxalacetic acid amide.

Many amino acids can be synthesized from the α -keto acids by virtue of this reaction: glycine, alanine, serine, cysteine, aminobutyric acid, norvaline, norleucine,

leucine, phenylalanine, tyrosine, tryptophane, methionine, glutamic acid, asparagine, arginine. The reaction does not occur with the α -keto acids of valine and isoleucine. It is possible that the two H-atoms at the β -carbon atom are essential if the reaction is to occur.

When the keto analog of arginine undergoes this reaction, the end products are urea, glutamine- γ -semi-aldehyde, and glutamic acid. Transamination probably first results in the production of arginine, α -ketoglutaric acid, and ammonia. Arginine is then broken down to ornithine and urea. Ornithine transfers its δ -amino group to the α -keto-glutaric acid, converting the latter into the γ -aldehyde and this into glutamic acid.

Ornithine can transfer its δ -amino group to glyoxylic acid to form glycine.

The reaction of the glutamine-transaminase system with β -mercapto-pyruvic acid results in the formation, not of cysteine, but of alanine; the sulfur is first broken off by a specific enzyme [189] before the amino group is transferred.

An asparagine-transaminase system probably also exists, for in the presence of α -keto acids, asparagine is rapidly deamidated [190]. This is however lacking in the kidneys. The transaminase involved is different from glutamine-transaminase. The resulting amide of oxalacetic acid is not hydrolyzed as rapidly as the amide of α -keto-glutaric acid and can, in addition, transaminate α -amino acids with the reappearance of asparagine.

In the presence of adenosine triphosphoric acid, glutamine is resynthesized from glutamic acid and ammonium salts [130, 191, 192].

The following 2 schemata (Fig. 71 a and 71 b) show the relationships between the 2 transaminase systems and other events in intermediate metabolism.

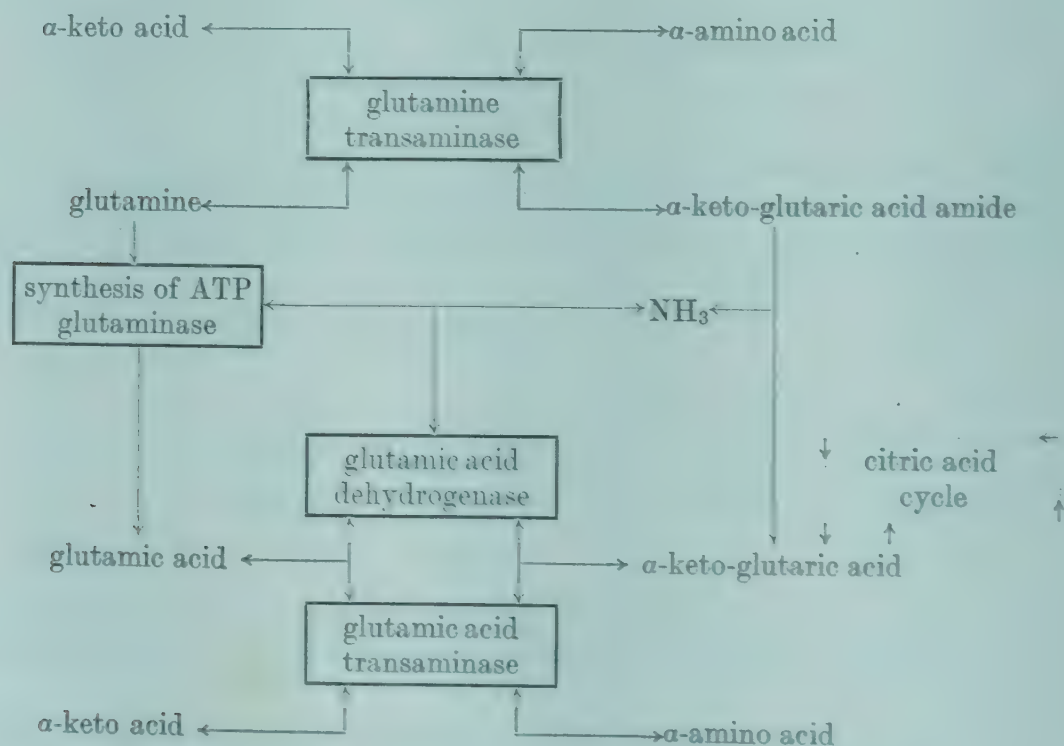


Fig. 71 a. The chief deamination and transamination reactions of glutamic acid, according to Meister.

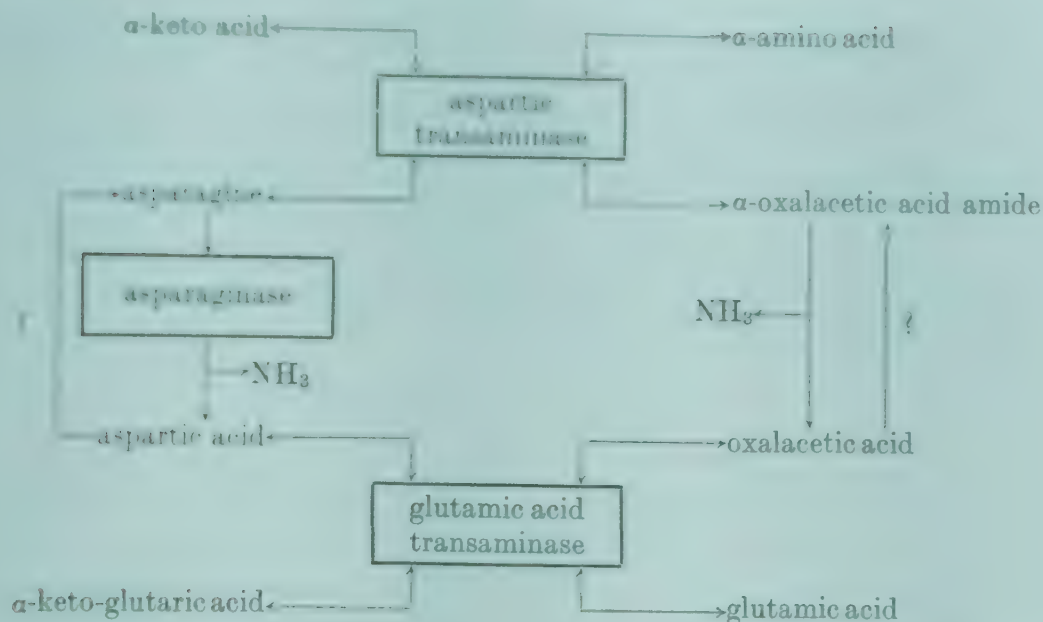


Fig. 71 b. The chief deamination and transamination reactions of aspartic acid, according to Meister.

Coenzymes involved in transamination are pyridoxal phosphate and pyridoxamine phosphate; they probably work in accordance with the following schema [193, 194]:

1. pyridoxal phosphate (or pyridoxamine phosphate) + enzyme \rightleftharpoons enzyme-pyridoxal-phosphate (or enzyme-pyridoxamine-phosphate)
2. enzyme-pyridoxamine-phosphate + α -keto-glutaric acid (or oxalacetic acid) \rightleftharpoons enzyme-pyridoxal-phosphate + glutamic acid (or aspartic acid).

The animal body is capable of synthesizing all non-essential amino acids. It accomplishes this synthesis either by utilizing the α -keto acids supplied by the citric acid cycle (α -keto-glutaric acid, oxalacetic acid) or by the degradation of glucose (pyruvic acid); or by converting one amino acid into another. With the aid of ammonia and a hydrogen donor, the α -keto acids undergo reductive amination (the opposite of oxidative deamination); or there is a simple interchange of amine between the keto acids and another amino acid.

The α -keto acids of the essential amino acids, with the exception of lysine, can, as already discussed (p. 366), undergo these same changes in growth experiments; that is, they can be converted into amino acids. The carbon skeleton must be supplied in the diet.

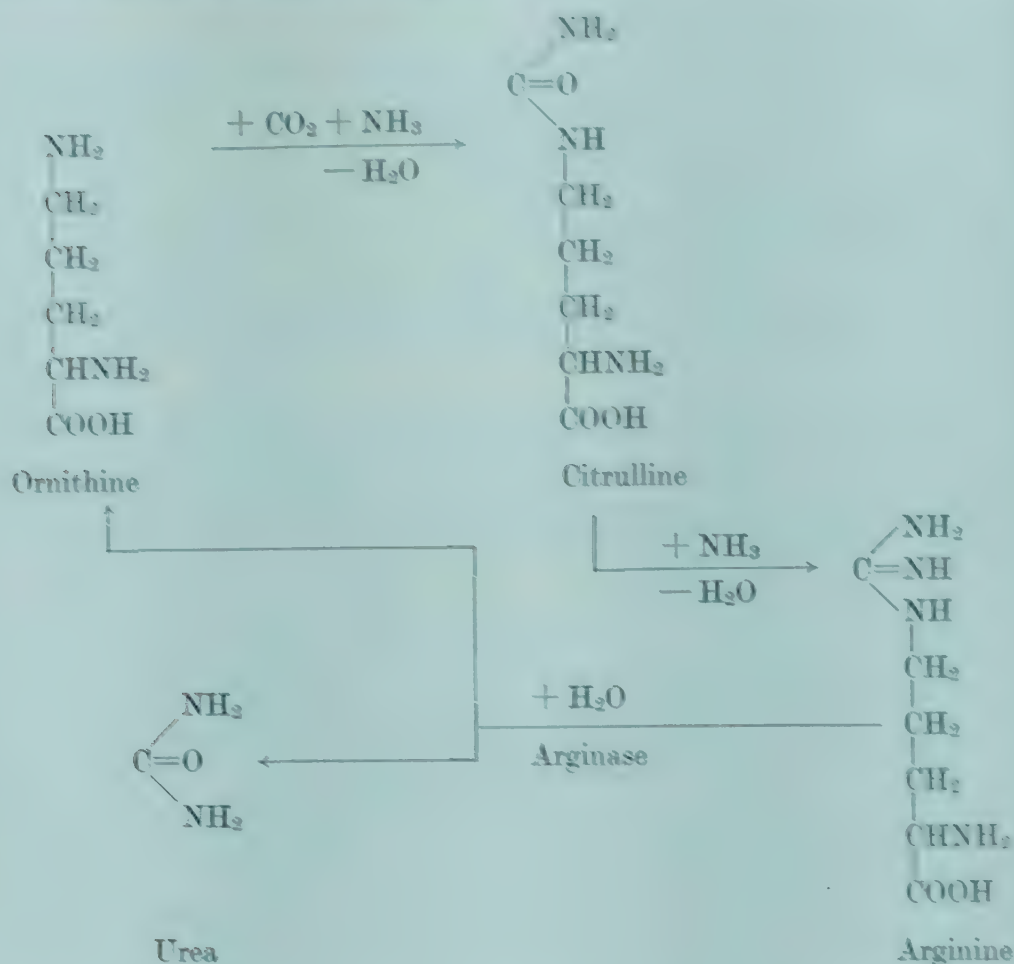
Finally, amino acids may be converted into one another. Serine may be converted into glycine, and glycine converted back into serine. Glutamic acid results from the degradation of proline, ornithine, and histidine. To a very small extent, lysine is oxidized to α -amino-adipic acid; ornithine can probably be changed into proline. The details of these conversions are discussed under the individual amino acids (p. 374).

Formation of Urea

In the preceding chapters, we have described how the amino group is removed from the amino acids. Ammonia results, either directly through oxidative deamination or indirectly through transamination with glutamine or asparagine. Only those animals which live in water are capable of excreting ammonia as such; all other animals con-

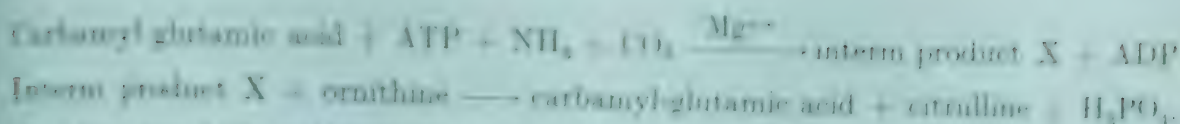
vert it into urea if they cannot use it directly in the amination of an α -keto acid or in the neutralization of an excessively acid urine. In birds, ammonia is first converted to urea, urea is then converted into uric acid, and uric acid is excreted as such.

Urea is synthesized by means of a cycle described by Krebs and Henseleit over 20 years ago in Thannhauser's clinic [195]. Ornithine, citrulline, and arginine are involved in this cycle. Arginine is split by arginase into urea and ornithine, and this can reenter the cycle anew. In this way, a small amount of ornithine can result in the synthesis of a large quantity of urea. In the cycle, 2 molecules of ammonia and 1 molecule of carbon dioxide are joined as follows:



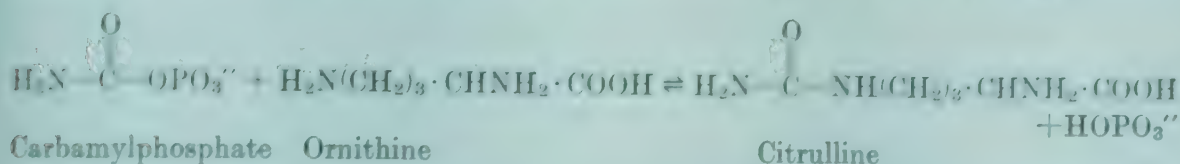
Urea can be formed only in those tissues in which arginase is present (in mammals and in man, in the liver).

In the last 10 years, experiments with tissue slices, tissue homogenates, cell fractions, and intact animals have allowed exact analysis of the individual steps of this cycle, and the mechanism of synthesis of citrulline and of its conversion into arginine has been extensively clarified. Citrulline is synthesized only in the presence of oxygen; or, in its absence, only if ATP is supplied. In addition to ammonia and carbon dioxide, rather large amounts of carbamyl-glutamic acid and magnesium ions must be present [196-198]. Carbamyl-glutamic acid reacts with carbon dioxide, ammonia, and phosphoric acid derived from ATP, to form a very labile intermediate product "X", which readily breaks down to carbamyl-glutamic acid, carbon dioxide, ammonia, and phosphoric acid [199]. This intermediate product now reacts with ornithine and forms citrulline; carbamyl-glutamic acid is reformed and phosphoric acid is set free.

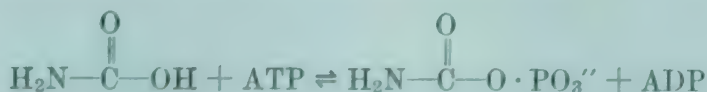


In these reactions, carbamyl-glutamic acid can be replaced by acetyl- or formyl glutamic acid and also by carbamyl-alanine [200, 200 a].

Jones, Spector, and Lipman [200 b] have shown, in experiments with acetone-dried powder of beef liver extract, that the synthesis of citrulline requires (in addition to ornithine, ATP, ammonia, and carbon dioxide), another cofactor which is a derivative of glutamic acid acylated at the nitrogen. They further found, however, that the same enzyme preparation synthesized citrulline from ornithine and carbamyl phosphate even in the absence of activating substances. Probably, carbamyl glutamic acid and the other activators first react with ATP to form carbamyl phosphate, which then reacts with ornithine. The exact course of the reaction is still to be determined. Carbamyl phosphate behaves like the intermediate product "X" and has various properties in common with this material. Grisolia et al., however doubt the identity of carbamyl phosphate with the intermediate product "X" [200 g].

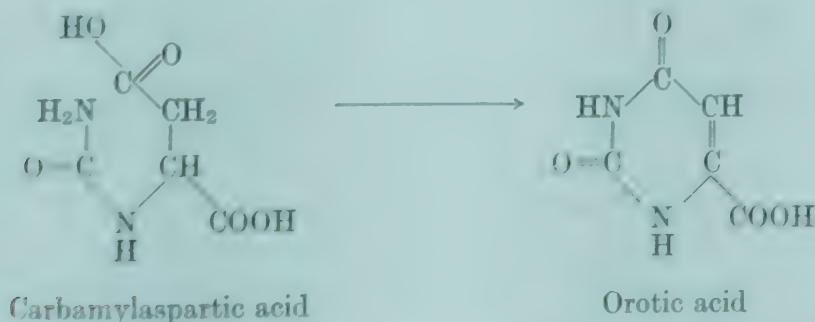


Bacteria (*Streptococcus fecalis*) synthesize citrulline in 2 steps. Carbamate is first phosphorylated by ATP and then the carbamyl radical of the resulting carbamyl phosphate is transferred to ornithine in accordance with the above equation.



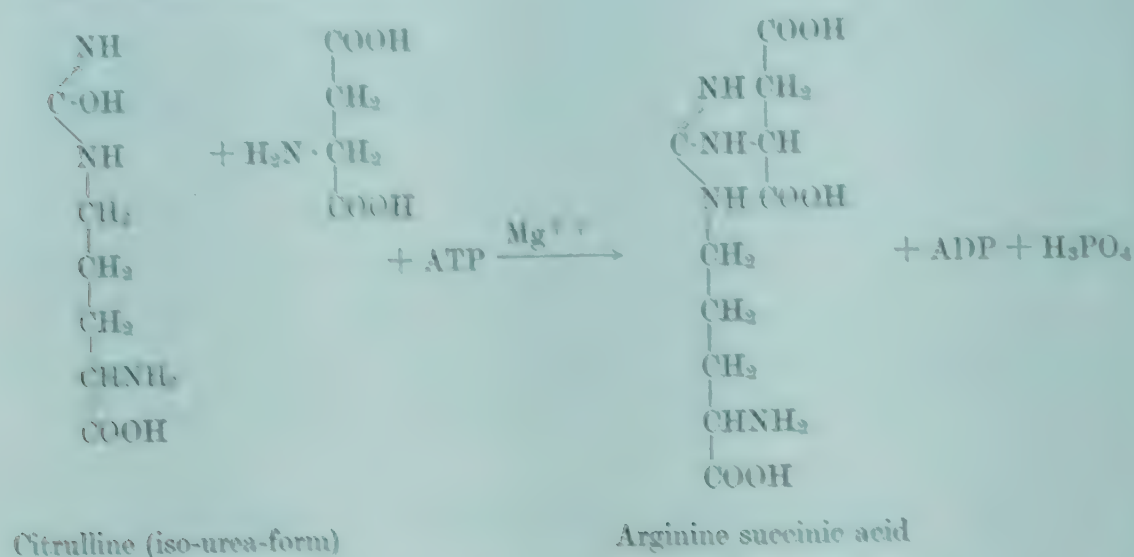
Both reactions are catalyzed by specific enzymes [200 b].

Carbamyl phosphate can also react with aspartic acid to form carbamyl-aspartic acid (ureido-succinic acid). This is a precursor in the synthesis of orotic acid, which is converted by animal tissues into uracil and cytosine. There is thus a relationship between the urea cycle and pyrimidine metabolism. The liver of rats can convert citrulline into orotic acid, probably by way of carbamyl phosphate and carbamyl aspartic acid [200 c-f].

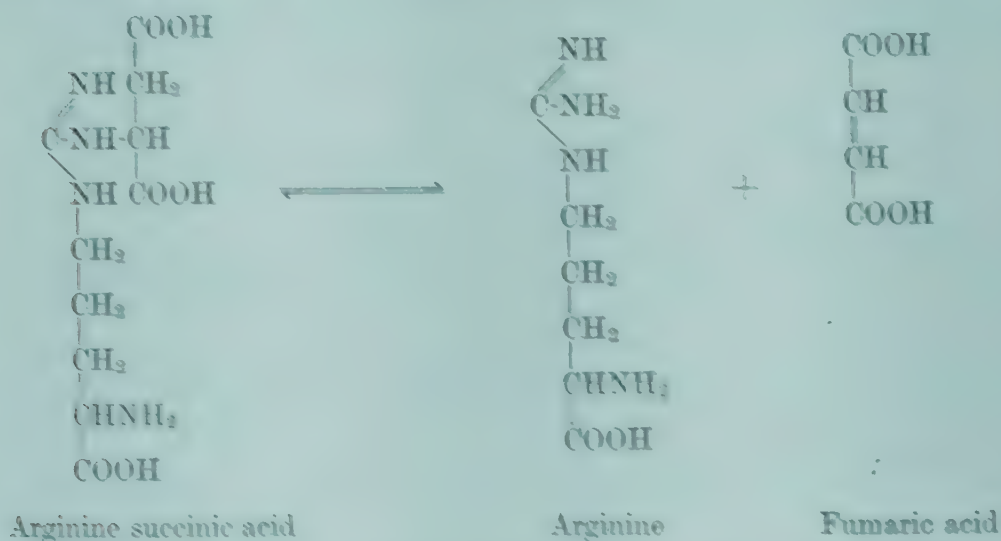


In the conversion of citrulline into arginine, aspartic acid, magnesium ions, and ATP again interact [201]. This process may merely involve a simple transamination in which citrulline takes over the function of an α -keto acid. The reaction, however,

is not reversible and uses up phosphate energy. The aspartic acid condenses with the iso-urea form of citrulline to form arginine-succinic acid [207]. In the process, the carbonyl carbon of citrulline is activated by ATP in a manner which is as yet unexplained. The activated product now reacts with aspartic acid with the liberation of inorganic phosphate.



Finally, the arginine-succinic acid, is broken down by a specific enzyme, arginine-succinase, into arginine and fumaric acid. This reaction is reversible.



In the conversion of citrulline to arginine, ammonia is involved only indirectly. It can first be bound by α -keto glutaric acid by reductive amination, for which hydrogen derived from reduced diphosphopyridine nucleotide is supplied. The resulting glutamic acid then transaminates with oxalacetic acid, giving aspartic acid, which then reacts with citrulline. The amino group which is liberated from the amino acids by the process of transamination can then be incorporated directly into urea by way of oxalacetic or aspartic acid respectively. Ammonia is taken up only when citrulline is synthesized.

The urea cycle is intimately connected with the citric acid cycle through α -keto glutaric acid and oxalacetic acid.

Fate of the nitrogen-free residue of the amino acids

With few exceptions the α -keto acids which are formed following decarboxylation or transamination of amino acids sooner or later, during their catabolism, enter the citric acid cycle. A few (for example, pyruvic acid derived from alanine) can be converted directly into glucose; others reach glucose only indirectly by way of the citric acid cycle. Still others (e. g., leucine, isoleucine, tyrosine) produce acetic acid before they enter the citric acid cycle and thus aggravate the symptoms of diabetes.

The details of the fate of the carbon structure are presented under the individual amino acids.

Decarboxylation of the amino acids

In animal tissues, only a fraction of the amino acids is decarboxylated; more decarboxylation occurs in plants and bacteria. In tissue cultures, Hultz and coworkers were the first to decarboxylate tyrosine, dihydroxyphenylalanine, and histidine [203]. The following table lists the amino acids which are decarboxylated by the animal, and the results of decarboxylation [204]:

serine	to colamine
cysteinic acid	to taurine
tyrosine	to tyramine
dihydroxy-phenyl-alanine	to hydroxy-tyramine
p-hydroxy phenyl-serine	to p-hydroxy phenyl ethanolamine (Arterenol)
5-hydroxy-tryptophane	to serotonin
histidine	to histamine
arginine	to agmatine
glutamic acid	to γ -amino-butyric acid
aspartic acid	to β -alanine

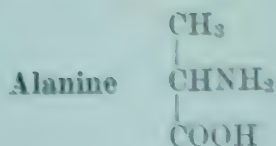
Various tissues, such as liver and kidneys, contain decarboxylases which are to a degree very specific for the individual amino acids and — perhaps with the exception of histidine decarboxylase — require pyridoxal phosphate as coenzyme [205].

Physiologically, decarboxylation is of importance in the synthesis of taurine, histamine, tyramine, arterenol, and serotonin. Taurine is a component of a bile acid. Histamine causes dilation of the capillaries. The other 3 amines act on the smooth muscle of the blood vessels and the intestine. The brain decarboxylates glutamic acid to γ -amino-butyric acid, which apparently has a specific function in the metabolism of nerve cells. Aspartic acid is decarboxylated to β -alanine, but only by bacteria. Putrescin and cadaverine are excreted in cystinuria, but must be synthesized by the intestinal flora from ornithine and lysine, for the necessary decarboxylases are lacking in the animal body. Some of the amines which are present in the intestines are among those substances which are the basis for the "auto-intoxication" sometimes attributed to the intestine.

Most amines can be further oxidized by way of the aldehyde to the corresponding carboxylic acids, but seldom does oxidation proceed beyond this stage. Among animal tissues, liver, kidneys, and brain are especially rich in decarboxylases. The cells at the base of the small intestinal crypts form serotonin [206].

The possibility that decarboxylation may be reversible has not been completely ruled out, so that amino acids may be resynthesized from the corresponding amines and carbon dioxide.

The Amino Acids and their Most Important Reactions



Both L-alanine and D-alanine are easily converted into pyruvic acid. L-alanine chiefly by transamination with α -ketoglutaric acid and to a lesser degree through oxidative deamination [173]. D-alanine, on the other hand, undergoes only oxidative deamination, the enzyme involved being D-aminoacid oxidase. According to metabolic requirements, the pyruvic acid can be converted to glucose and oxidized to acetic acid, and can then enter into the citric acid cycle, or can be utilized for the synthesis of fatty acids and cholesterol. In addition, it can be carboxylated to oxalacetic acid and then either condensed to citric acid with acetic acid derived from the β -oxidation of fatty acids, or aminated to aspartic acid with the help of ammonia. Alanine is thus not only the best source of glucose among the amino acids, but can also support other important metabolic processes.

Alanine is not an essential amino acid, for it can be easily synthesized from pyruvic acid. However, it is present in almost all proteins.

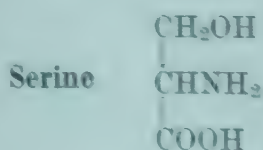


New experiments with C^{14} -labelled glycine have shown that this simplest of all amino acids is involved in many metabolic processes. As already discussed, there is a specific yellow enzyme present in many animal tissues, especially in the liver and kidneys of mammals, which deaminates glycine to glyoxalic acid [169]. Under physiological circumstances, however, this enzyme seems to have little opportunity to act, for liver slices form little urea and ammonia from glycine [207].

In vitro, glyoxalic acid which results from the deamination of glycine is first oxidized to formic acid and carbon dioxide. The formic acid is then oxidized to carbon dioxide in a coupled reaction, or utilized in the form of C_1 -bodies for synthesis. If there is much glyoxalic acid present, some of it is converted into oxalic acid, which cannot be further broken down [208].

Glycine can be resynthesized from glyoxalic acid and glycolic acid [208].

Glycine reacts with benzoic acid to form hippuric acid and with cholic acid to form glycocholic acid, and can be incorporated into the porphyrin and purine rings. Methylation of glycine results in sarcosine and betaine. Amidation of glycine produces guanidino-acetic acid; coupled methylation then results in the formation of creatine. Glycine may perhaps be reduced to colamine. Glycine is very easily converted to serine (see below) by reversal of the process by which it is formed from serine: addition of formic acid with accompanying reduction. Glycine can also be formed indirectly from serine through colamine (p. 375). Its transformation into glucose is by way of serine and pyruvic acid.



This is the most simple hydroxy-amino acid (2-hydroxy- α -amino propionic acid), and is as widely distributed among proteins as is alanine. In the phosphoproteins casein and vitelline, serine is present as its phosphonic acid ester. It is also a building stone of the serine-cephalins.



The β -hydroxy-amino acids do not undergo deamination in the same manner as the simple amino acids, but as a rule are first oxidized at the carbon atom which carries the hydroxyl group. Knoop has concluded that glycine must be formed in this way from serine [209]. We have already discussed the close interrelationships between these 2 amino acids. Shemin performed experiments in rats and guinea pigs using serine labelled with N^{15} and C^{13} (in the carboxyl group) [210], and was able to furnish direct proof for the transfer of the 1st and 2nd carbon atoms and the nitrogen of serine into glycine. The β -carbon atom is split off as formic acid; folic acid is necessary for this reaction. When rats are fed a diet deficient in folic acid, they synthesize less glycine from serine [211]. The vitamin binds the liberated formic acid.

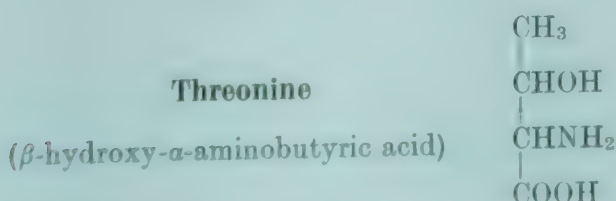
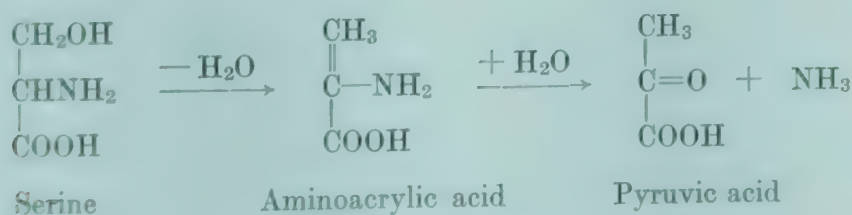
Because of this reaction, serine constitutes one of the sources of supply of one-carbon bodies, which are necessary in various syntheses. Thus, the β -carbon atom appears, under particular circumstances, in the methyl groups of choline [212, 213].

The synthesis of serine from glycine and formic acid is the reverse of this reaction [214]. However, the reaction seldom proceeds in this direction. Formic acid can come from methyl groups from methionine or choline, or can be formed from glycine itself. In the latter instance, 2 molecules of glycine are necessary for the synthesis of serine. One of these must be broken down to formic acid and carbon dioxide. The formic acid is then again transferred, by means of folic acid, to the other glycine; an intermediate product is formyl-folic acid. ATP must be present for this process.

As has already been stated, serine can undergo decarboxylation to form colamine (ethanolamine) [215, 216].

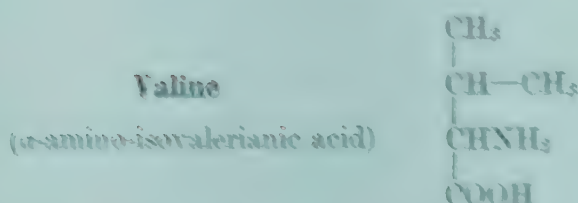
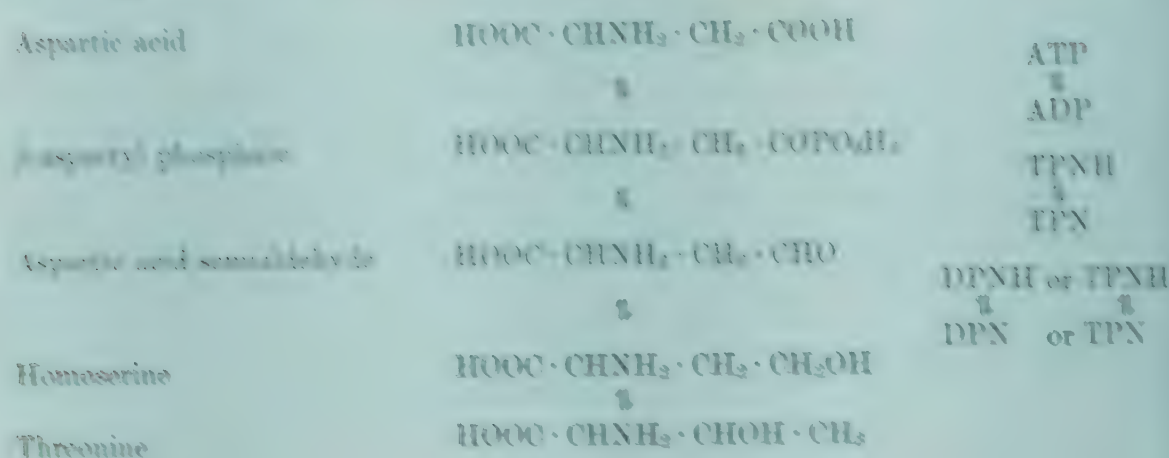
Finally, serine can also be converted to pyruvic acid [217]. Probably, water is split off and amino-acrylic acid is formed, which is immediately broken down to pyruvic acid and ammonia.

This degradation process relates the metabolism of serine to carbohydrate metabolism and to the citric acid cycle:

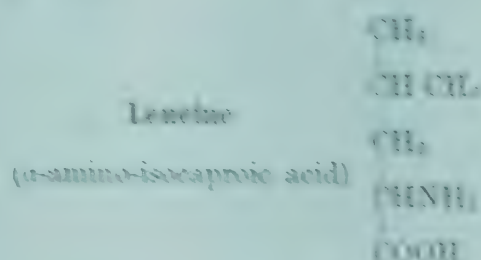


Threonine is an essential amino acid. Its degradation in the body is not yet exactly known. It probably undergoes oxidation to form glycine and acetic acid [206]. Bacteria break threonine down in a manner similar to their action on serine. Probably, water is first split off and α -amino-crotonic acid formed, which then breaks down into α -ketobutyric acid and ammonia [217].

Bacteria produce threonine from aspartic acid. Intermediate products are aspartyl- β -phosphate, aspartic acid- β -semialdehyde, and homoserine [218], as shown in the following schema:

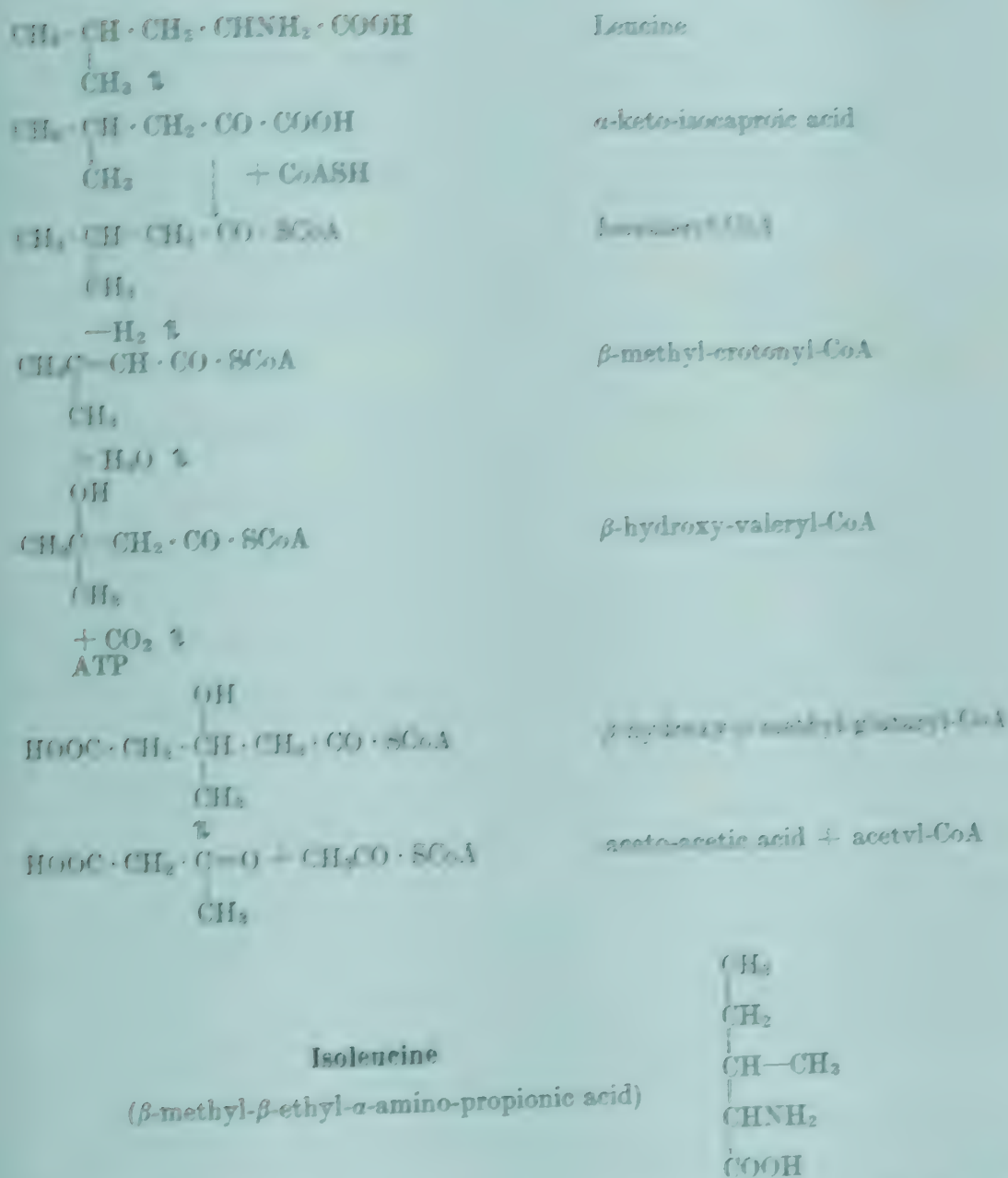


Valine is also an essential amino acid. When it is lacking in the diet, the animal shows characteristic symptoms involving the central nervous system. Three of the 5 carbon atoms, probably those of the isopropyl radical, can be converted into glucose [219]. Since α -ketoisovalerianic acid and isobutyric acid likewise change over into glucose, valine must first be transformed into the corresponding keto acid, probably by transamination with α -keto-glutaric acid [173, 218, 220], perhaps also by direct oxidative deamination. Certainly L-aminoacid oxidase reacts very slowly with valine [173]. The α -keto-isovalerianic acid can replace valine in diet experiments, and can thus be aminated [221, 222].



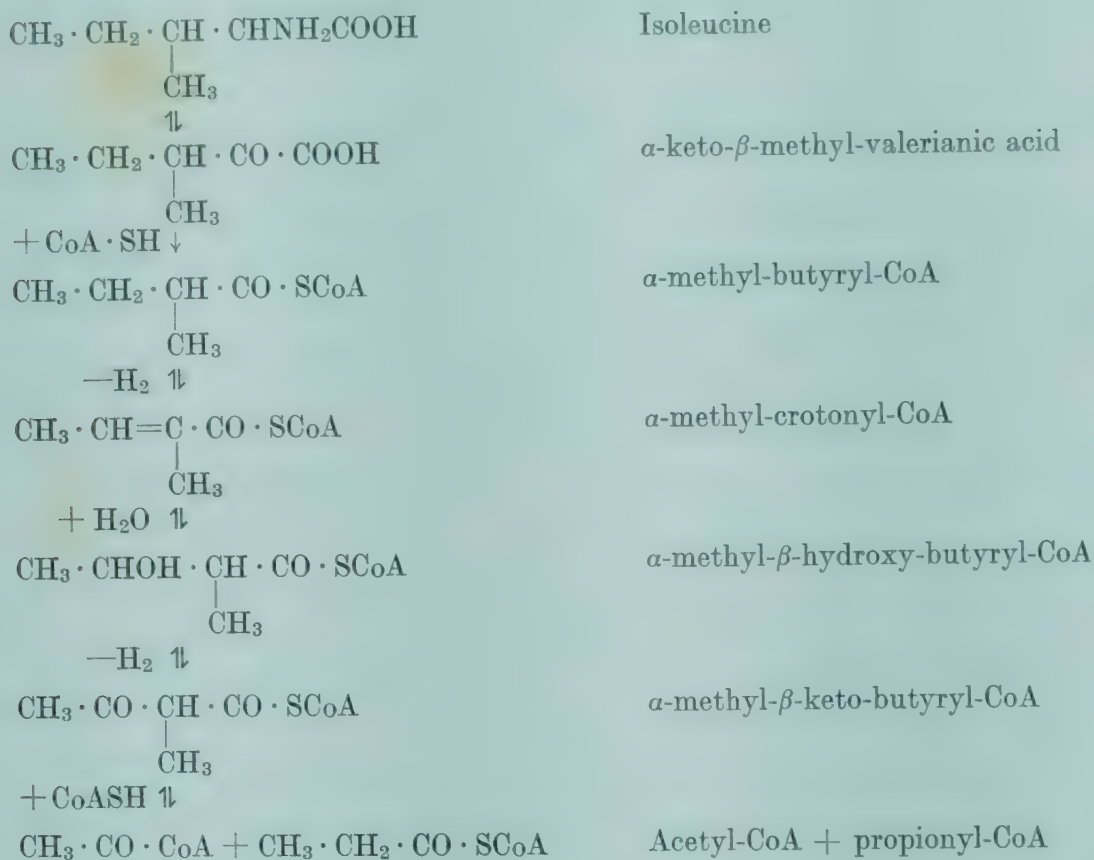
Leucine, an essential amino acid, is the strongest ketogen [223]. During degradation like valine, it undergoes transamination with α -keto-glutaric acid (or, in the liver with pyruvic acid) and is converted into α -keto-isocaproic acid [218, 220]. Leucine can also be deaminated by L-aminoacid oxidase.

The α -keto-isocaproic acid is oxidized to isovalerianic acid. Experiments by various authors [214] have shown that a molecule of carbon dioxide is fixed in the pathway which leads to acetic acid. The α -keto-isocaproic acid, according to the view of these authors, undergoes oxidative decarboxylation to form the coenzyme A ester of isovalerianic acid, and this undergoes dehydrogenation and addition of water to form the corresponding β -hydroxy-derivative. Interaction with ATP results in the addition of carbon dioxide at the end methyl group. This follows degradation to acetyl-coenzyme A and acetic acid, whose carboxyl group is thought to come from the fixed carbon dioxide. The following scheme gives the probable course of degradation of leucine:



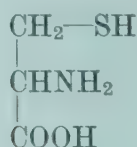
Isolencine, also an essential amino acid, is only a poor nitrogen, but, on the contrary is a distinct producer of glucose. Its catabolism is similar to that of leucine; first there is transamination to the corresponding keto-acid, then there is formation

of α -methyl-butryl-coenzyme A, then α , β -dehydrogenation, addition of water, and transformation into β -hydroxy- α -methyl-butryl-coenzyme A; then dehydrogenation by means of diphospho-pyridine-dinucleotide to the corresponding β -keto-acid; and, finally, splitting by means of a second molecule of coenzyme A into acetyl-coenzyme A and propionyl-coenzyme A. Two molecules of acetyl-coenzyme A can form acetoacetic acid [225]. The propionic acid of propionyl-coenzyme A may be converted into glucose.



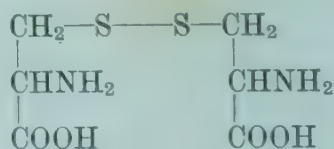
Cysteine

(β -thio- α -amino-propionic acid)



Cystine

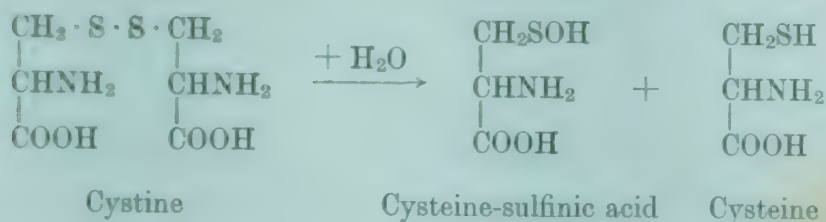
(di-cysteine)



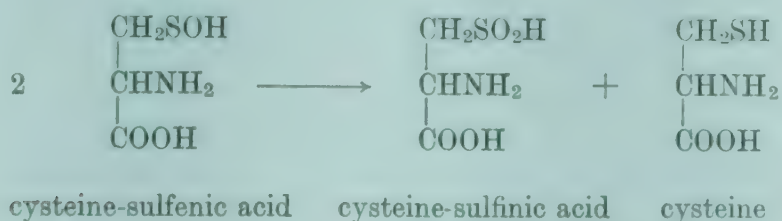
In the body, cysteine is readily oxidized by cytochrome C and cytochrome oxidase to cystine [226]. Respiratory poisons such as cyanide, sodium sulfide, and carbon monoxide inhibit this oxidation. The oxidation can also be accomplished by elemental sulfur, the sulfur being converted into hydrogen sulfide.

Conversely, cystine is reduced to cysteine by the action of the reduced form of diphospho-pyridine-dinucleotide, at least in yeast and the higher plants.

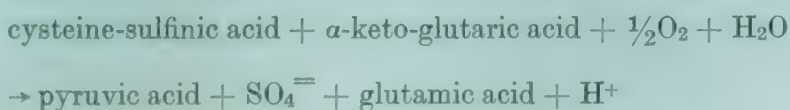
Since the two amino acids can be readily converted into each other, their catabolism is the same. As a rule, cystine is transformed into cysteine before the oxidation of the carbon chain and of the sulfur. However, cystine can also be broken down enzymatically through the addition of water into cysteine-sulfenic acid and cysteine:



The sulfur-containing end product of the oxidation of cystine is the sulfate. The process of oxidation usually begins at the sulfhydryl group. The first phase is not as yet clear; probably, cysteine is oxidized to cysteine-sulfenic acid, which is rapidly converted to cysteine-sulfinic acid; perhaps 2 molecules of cysteine-sulfenic acid react to form cysteine-sulfinic acid and cysteine [227, 228, 229]:

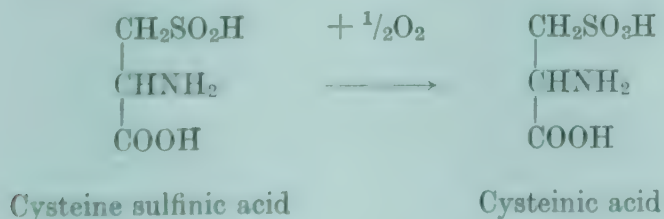


Cysteine-sulfinic acid is rapidly broken down by animal tissues (livers of rats and rabbits) and by bacteria into sulfate and pyruvic acid, and eventually also to alanine. A portion is also decarboxylated to form hypotaurine [230-233]. The corresponding enzymes are found in the mitochondria of the liver cells [23]. The amino group is removed by transamination with α -keto-glutaric acid or oxalacetic acid. Oxalacetic acid can be replaced by fumaric acid or malic acid, in the presence of additional diphospho-pyridine-dinucleotide, probably according to the following mechanism:



Pyruvic acid can react further with glutamic acid, giving alanine and re-forming α -ketoglutaric acid. In addition, the possibility exists that cysteine-sulfinic-acid can be converted directly to sulfin-pyruvic acid by oxidative deamination [235].

Taurine can be formed in 2 ways: either cysteine-sulfinic acid is oxidized to cysteinic acid and decarboxylated at the same time; or it is decarboxylated to hypotaurine and the latter then oxidized to taurine [236, 237]:





Methionine

(α -amino- γ -methyl-thiol-n-butyric acid)

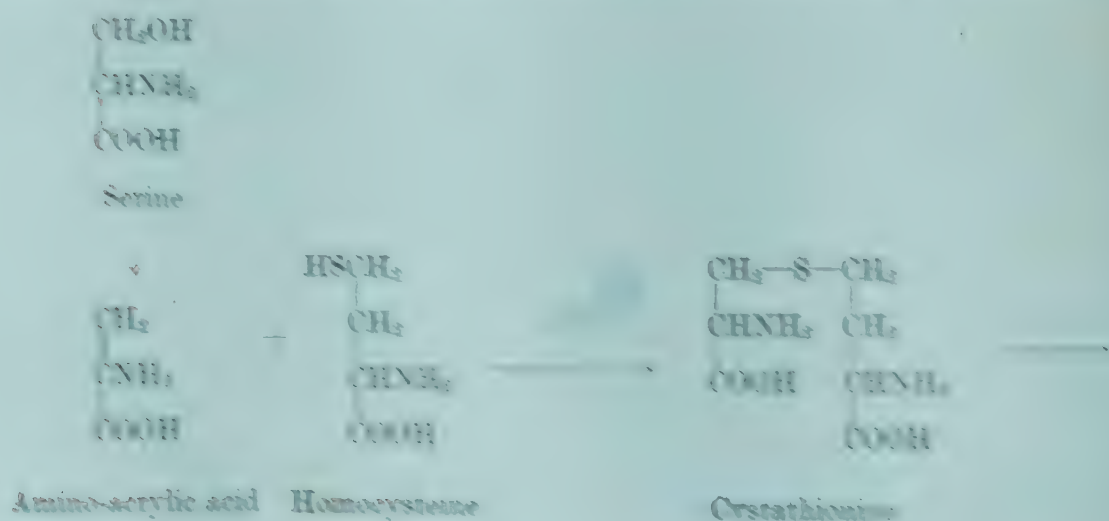


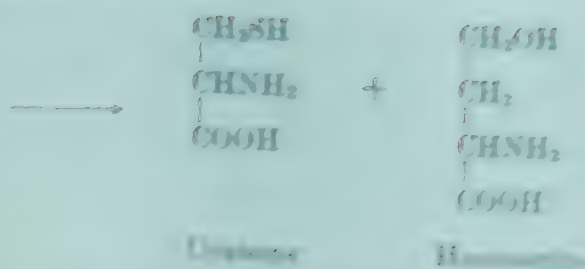
The importance of methionine in metabolism consists of the fact that it is involved in the synthesis of cysteine and in the methylations in the body.

When methionine is administered to patients with cystinuria, their urine shows increased excretion of cystine [238]. When it is fed to rabbits together with bromobenzol, the urine shows mercapturic acid [239]. As already shown, only the sulfur of methionine is transferred to cysteine.

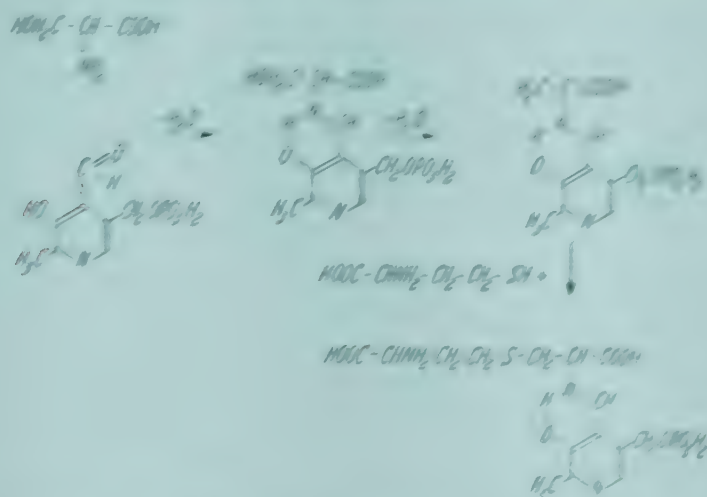
The synthesis of cystine from methionine takes place chiefly in the liver. Liver preparations actually form very little cystine when methionine is added to them. If, however, homocysteine and serine are added to the same liver preparations, about 60% of the sulfur appears in cystine [240]. When methionine labeled with S^{35} and C^{14} in the β and γ locations is fed to rats, the isolated cystine contains only S^{35} , while the carbon atoms of the cystine are not radioactive [241]. This fact confirms that it is only the sulfur of methionine which passes into the cystine; the carbon and nitrogen come from serine.

First, methionine must undergo demethylation and be converted into homocysteine. At the same time, serine is probably dehydrated to amino-acrylic acid, which condenses with homocysteine to form cystathionine. This is broken down by a thionase into cysteine and homoserine. Cystathionine can replace cystine, but not methionine, in the diet of growing rats; this is a sign that homocysteine cannot be split off from cystathionine. The following schema gives the transformation of homocysteine to cysteine:

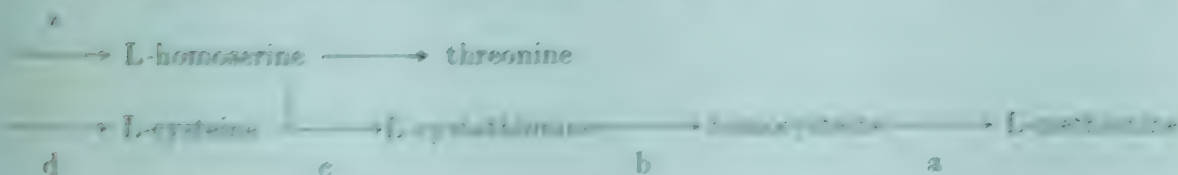




Pyridoxal phosphate is probably involved in this process. It probably binds amino acids in the manner of a Schiff's base, and in this combination some is oxidized. In this way, the degradation of amino acids and directly non-protein acid and ammonia is initiated. Homocysteine is then added at the double bond. Finally, the pyridoxal phosphate must again be split off by hydrolysis [232]:



Animals for which methionine is an essential amino acid are incapable of splitting cystathionine in such a way as to re-form homocysteine. Various micro-organisms, however, contain the requisite enzymes, and so are capable both of synthesizing cystathionine from cysteine and homoserine, and of breaking down cystathionine into serine and homocysteine. Experiments with mutants of *Saccharomyces crassus* have shown that the individual steps in the transformation of cysteine to methionine are controlled by specific genes (in the following schema, these are shown in small letters) (143):



If one of these genes is lacking in a mutant, the reaction is interrupted, and the amount of the subsequent material increases, for the absence of the gene means the absence of the corresponding enzyme which catalyzes the reaction. According to the one gene-one enzyme theory, a specific gene corresponds to each enzyme.

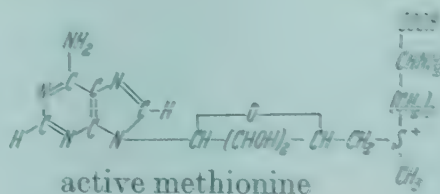
The sulfur of cysteine in the last analysis comes from inorganic sulfate, which is reduced, chiefly by bacterial action, to hydrogen sulfide. The intermediate is aminocaproic acid, with the formation of cysteine. Aminocaproic acid is produced as discussed above, from aspartic by the loss of a molecule of water through the action of pyridoxaldehyde.

It is of interest that radioactive sulfur appears in the methionine and cystine of the milk of goats, sheep, and cows, when they are fed $\text{Na}_2\text{S}^{32}\text{O}_4$ [244, 245]. Under such conditions, hens lay eggs whose cystine contains S^{32} [246]. In these cases, it may be that the intestinal bacteria have reduced the sulfate. Rabbits fed in this way incorporated S^{32} in the cystine of their tissue proteins, even though they were prevented from eating their feces [247].

Transmethylation and other reactions of the methyl groups

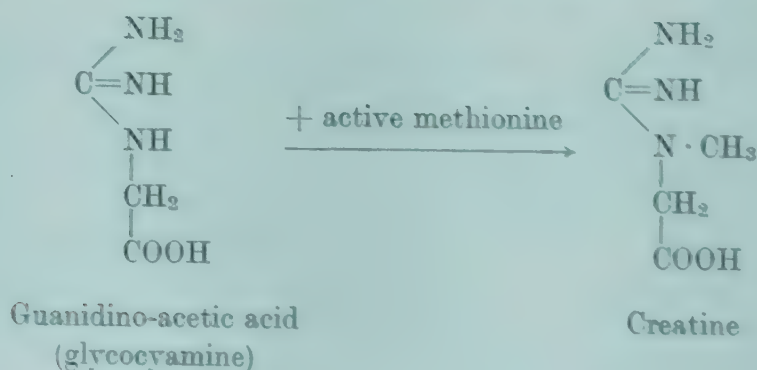
If methionine is to supply its sulfur for the synthesis of cysteine, the methyl group must first be split off. This event is of general importance in animals and plants, for it supplies the methyl groups for the conversion of colamine into choline, of guanidino-acetic acid into creatine, of carnosine into anserine, of glycine into sarcosine and betaine, of arterenol into adrenalin, of nicotinic acid into trigonelline, etc. — i. e., for *transmethylation*. Homocysteine is formed as a side reaction and can be used in the synthesis of cysteine or can be converted back into methionine. The analysis of this event at the same time gives information on the first step of cysteine synthesis and the last step of methionine synthesis. Further information is needed concerning how the methyl group is supplied. Furthermore, since methyl groups can also be released by oxidation (i. e., as formaldehyde or formic acid), there is a close relationship between the metabolism of methionine and that of C_1 -bodies.

In the methylation of guanidinoacetic acid to creatine, two separate enzyme reactions must be distinguished [248, 249, 250]. An activating enzyme converts methionine in the presence of ATP into "active methionine." Magnesium ions and glutathione must be present. The active methionine is said to be a compound of methionine and adenosine, as in the following provisional formula. The adenosine portion is apparently supplied by ATP.

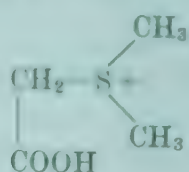


According to this formula, adenosine-methionine is a sulfonium compound with positively charged sulfur.

A second enzyme transfers the labile methyl group to the guanidino-acetic acid, as follows:



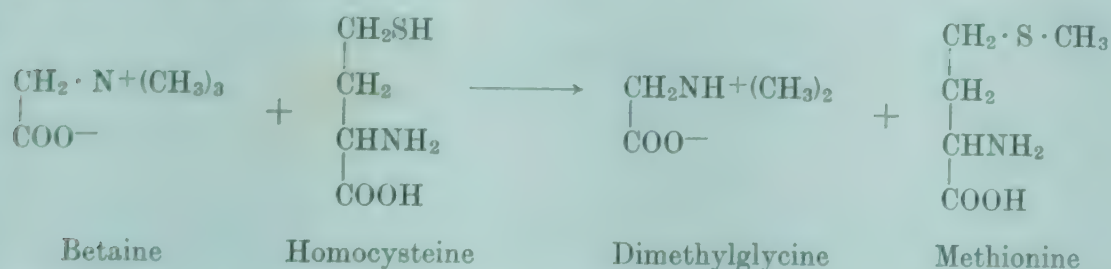
Synthetic sulfonium compounds can replace methionine as methyl donors in the growth test in animals. The formula of one of these, dimethylthetine, is as follows.



Dimethylthetine

A similar reaction apparently occurs in the methylation of colamine to choline; 3 molecules of methionine are necessary in this process. The same reaction may hold for the other methyl compounds discussed above.

For the *regeneration* of the *methyl groups* of methionine, choline and betaine are of greatest importance [251]. Thus in the presence of oxygen, liver slices and homogenates can transfer one or 2 of the methyl groups (not the third) from choline to homocysteine. Choline is not active under anaerobic conditions but betaine remains active [252, 253]. Betaine may thus be the particular donor which results from the oxidation of choline. Liver contains a choline oxidase. An intermediate betainealdehyde is postulated in the reaction [254]:



What additional substances are involved in this reaction is not known.

Sarcosine, which results from the successive demethylation of betaine, can also be synthesized from glycine and methionine.

The methyl groups of betaine can also be utilized for the methylation of guanidinoacetic acid, but only in the presence of homocysteine. Methionine may perhaps be formed as an intermediate substance.

The methyl group of creatine is not labile and can no longer be used for the synthesis of methionine or choline.

As already explained, the *methyl group* of methionine can also be liberated by oxidation. When C^{14} -labelled methionine (C^{14} in the methyl group) is fed to rats, the exhaled air contains C^{14} -carbon dioxide [255, 256]. The same is true for choline. The carbon of the methyl group is found not only in the expired carbon dioxide, but also in the β -C atom of serine [257]. The oxidation of the methyl group can thus stop at the stage of formaldehyde and formic acid. These " C_1 -bodies" can be used in syntheses to the degree that they are not excreted or completely oxidized: for example, in the synthesis of serine and of purine nucleotides.

Conversely, the methyl group can be regenerated from formic acid and formaldehyde [258, 261]. The hydrogen comes partly from the water of body fluids. Most of the methyl group used in transmethylation comes, however, from the diet.

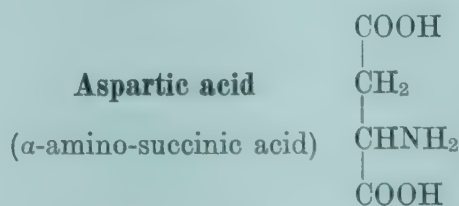
When the diet of young rats lacks labile methyl, but homocysteine is fed, the rats stop growing, their livers show fatty infiltration (deficiency of choline), and their

kidneys show hemorrhagic degeneration. Addition of folic acid and vitamin B₁₂ to the diet prevents these changes. Thus, these vitamins seem to be concerned with the formation of methyl groups, the synthesis of methionine and choline, and transmethylation, respectively.

Rats deficient in folic acid are less capable of transferring the methyl groups of betaine to homocysteine, colamine, and guanidino acetic acid, or of incorporation of their carbon into serine. Also, the conversion of methionine to colamine is impaired [262]. The relationship of folic acid to transmethylation is not clear. In the synthesis of serine, it may be that, in the absence of folic acid, the methyl group can no longer be oxidized to formaldehyde and formic acid. When folic acid is lacking, purine synthesis is also faulty; this synthesis may also be indirectly bound to transmethylation [261]. In the absence of folic acid, the reduction of formaldehyde and formic acid to methyl is also inhibited [261].

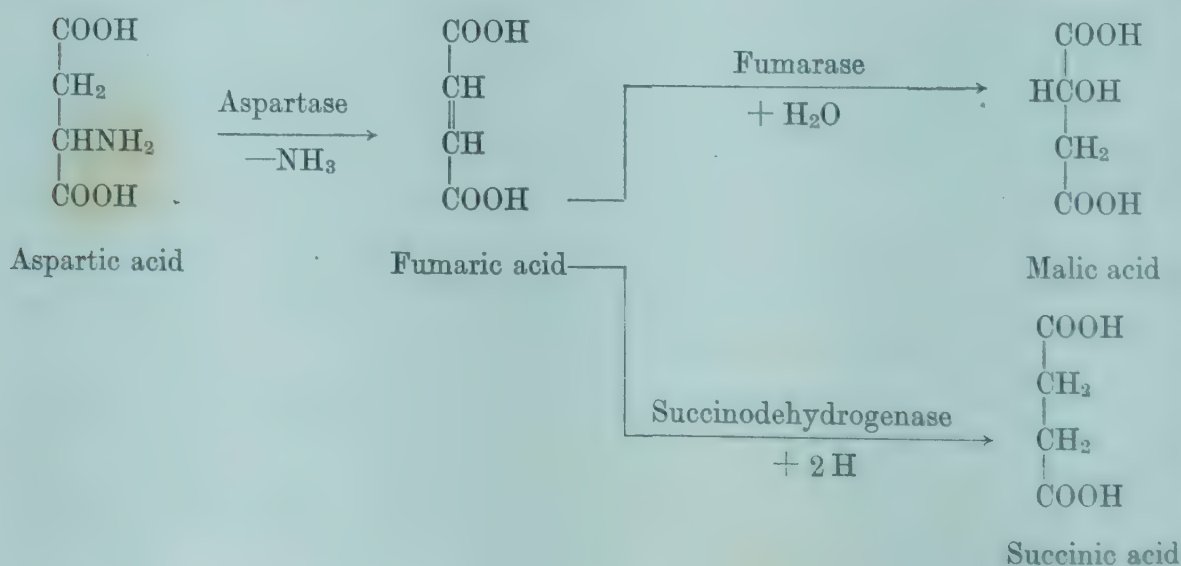
In a few experiments in young rats fed on a diet lacking in vitamin B₁₂, it was found that there was difficulty of incorporation of the carbon of formic acid, of the α -carbon from glycine, and of the β -carbon from serine into the methyl groups of choline and methionine.

Vitamin B₁₂ is also necessary for the methylation of guanidino-acetic acid into creatine [263].



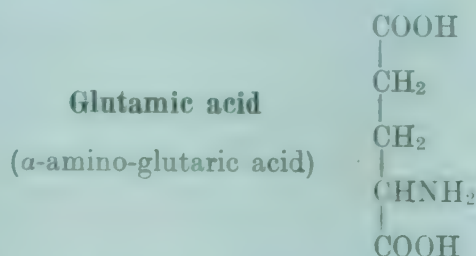
The known facts concerning the metabolism of aspartic acid were covered in the section on transamination. Transamination with α -keto-glutaric acid converts aspartic acid into oxalacetic acid, which can immediately enter the citric acid cycle or can be decarboxylated to pyruvic acid.

Various bacteria (e. g., *E. coli*) contain an enzyme, aspartase, which breaks aspartic acid down to fumaric acid and ammonia [264, 265]. Fumaric acid can be converted by fumarase into malic acid, or can be reduced by succino-dehydrogenase to succinic acid. All 3 dicarboxylic acids are components of the citric acid cycle.



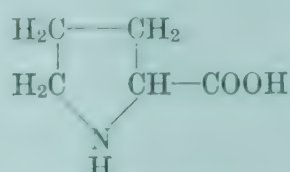
The nitrogen which is introduced into the body of an animal with aspartic acid becomes, by transamination, rapidly available to the body for the synthesis of other amino acids.

Splitting off of the α -carboxyl group of aspartic acid converts it to β -alanine, which is present in carnosine, anserine and coenzyme A.

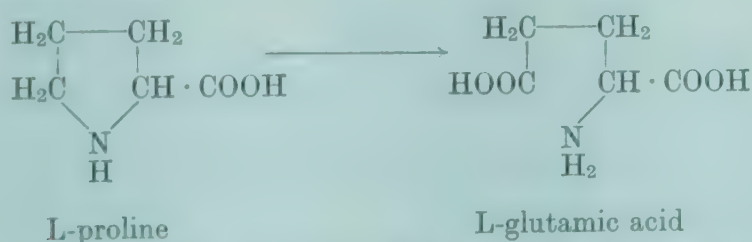


Glutamic acid also is chiefly transaminated into α -keto-glutaric acid and can thus promptly enter the citric acid cycle. As already discussed above, it may also be converted to glutamine. Glutamic acid plays a special role in the central nervous system. Through decarboxylation, it is converted into γ -amino-butyric acid. Besides this, it also serves to bind the ammonia which is found in the nerve cell [266]. It may also help to supply potassium ions to the nerve cells [267, 268].

Proline
(α -pyrrolidine carboxylic acid)



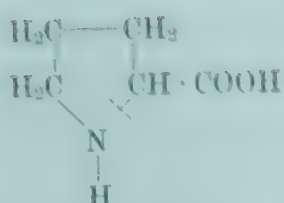
Liver and kidney slices of a variety of animals convert L-proline into glutamic acid and this in turn to α -keto-glutaric acid [165, 269–273]. In this way, the catabolism of this cyclic imino acid also is related to the citric acid cycle.



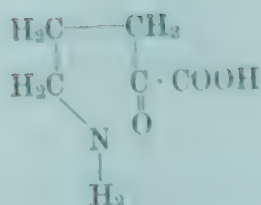
Nothing definite is known concerning the enzymes which are involved in the conversion of L-proline into glutamic acid or concerning the intermediate products which are formed. It is possible that the first step is dehydrogenation between C-5 and the imino group, and then water is so affixed that a hydroxyl group results at the C-5 carbon atom. This hydroxyl group could then be dehydrogenated to a carbonyl group and then the ring opened by hydrolysis.

Three carbon atoms of L-proline can be converted to glucose. This conversion probably occurs by way of α ketoglutaric acid, oxalacetic acid, and pyruvic acid.

In the case of proline, the different reactions of the 2 optical antipodes of an amino acid can be especially well demonstrated. D-proline is oxidized to α -keto- δ amino valerianic acid by means of the enzyme D amino acid-oxidase [274–276].



D-proline

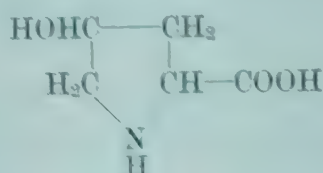
 α -keto- δ -amino-valerianic acid

The oxidation proceeds as in the other D-amino acids, except that the amino group at the 5th carbon remains and is not set free as ammonia.

In the rat, proline can serve as a precursor of ornithine and thus also of arginine [30, 276]. In this way, the rat can synthesize arginine in limited amounts, but not at a sufficiently rapid rate to cover the requirements of growth. Conversely, ornithine can be converted to proline.

Hydroxyproline

(γ -hydroxy- α -pyrrolidine-carboxylic acid)

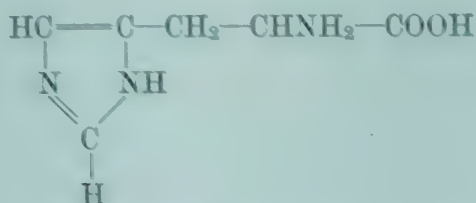


Labelled hydroxyproline which is ingested is not incorporated into body protein to any notable degree [277] and is largely excreted in unaltered form [278]. When, on the contrary, labelled proline is fed, definite amounts of labelled hydroxyproline are also demonstrable in the tissue proteins. Hydroxyproline thus is formed from proline, but can no longer be reversibly transformed into proline.

Hydroxyproline is also broken down to glutamic acid. Liver slices, however, are said to convert it into ketone bodies.

Histidine

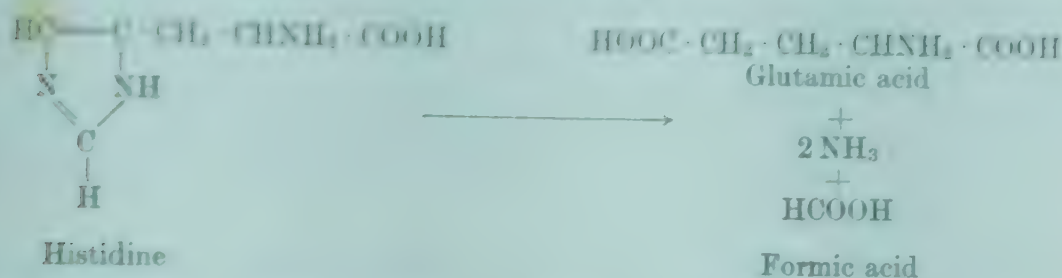
(α -amino- β -imidazol-propionic acid)



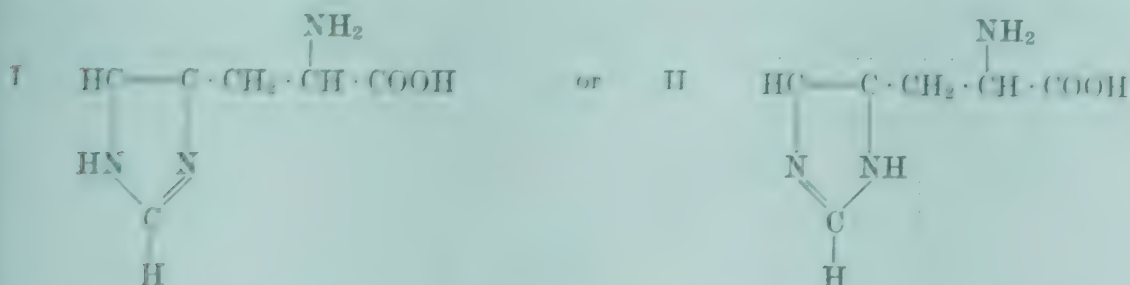
Histidine is an essential amino acid for all animals and for growing human beings. When it is absent from the diet of the adult, the nitrogen balance of the individual is actually undisturbed, but it is not known whether the individual can really synthesize it himself, or if he obtains it from the intestinal bacteria [20].

In feeding experiments in young rats, L-histidine can be replaced by α -acetyl-histidine, D-histidine, imidazol-lactic acid and imidazol-pyruvic acid. Thus, it is the skeleton of the molecule which cannot be synthesized: the amino groups themselves can be added by the rat.

In normal metabolism, histidine can be readily broken down to carbon dioxide, ammonia, and water [279]. The other materials which are formed in this process have been investigated by Kellbäcker et al. [280]. He found that histidine is broken down by an enzyme system, histidase, which is found in the liver of various animals, into glutamic acid, formic acid, and 2 molecules of NH_3 .



The glutamic acid is derived from the side chains and both of the upper carbon atoms of the imidazol ring. It must not, however, be immediately assumed that the α -amino group of the alanine residue becomes that of the glutamic acid. If this were so, both molecules of ammonia would have to come from the ring. It is just as likely that the α -amino group is split off as ammonia; then only one of the 2 nitrogen atoms of the imidazol ring (the left one) would be converted to ammonia, and the other nitrogen atom would pass to the α -amino groups of the glutamic acid. The carboxyl group of histidine would then become, not the α -, but the γ -carboxyl group of the glutamic acid. Both of these possibilities are depicted in the following formulas [281].

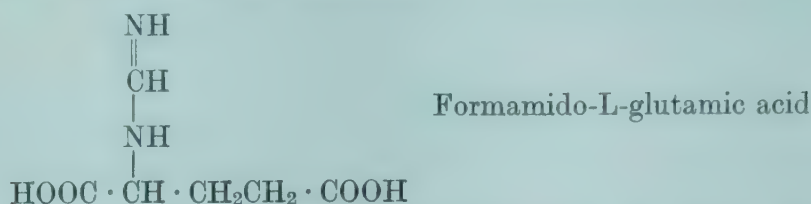


It became possible to assume the second of these alternatives as a result of observations of various authors that dogs excrete *urocanic acid* when they are fed histidine [282-285]. Urocanic acid is imidazol-acrylic acid [286]. Urocanic acid results because ammonia is split off from histidine without oxidation. It is also converted in liver broth to glutamic acid [280], in which process only one molecule of ammonia and also one molecule of formic acid occur.

The differentiation between the 2 alternatives was accomplished by experiments with cultures of fluorescent *Pseudomonas*, which also breaks down histidine into glutamic acid, ammonia, and formic acid [281]. When histidine was supplied which contained N^{15} either in the amino group or in the γ - or δ -nitrogen, the amino group of the resulting glutamic acid showed N^{15} only when the γ -nitrogen of histidine was labelled. N^{15} in the α -amino group or at the δ -nitrogen was found to appear in the ammonia. Identical results were found in experiments with urocanic acid labelled with N^{15} in the γ - and δ -locations.

Thus, the first product formed in the breakdown of histidine is urocanic acid. Assumption II above gives the actual sequence of histidine degradation.

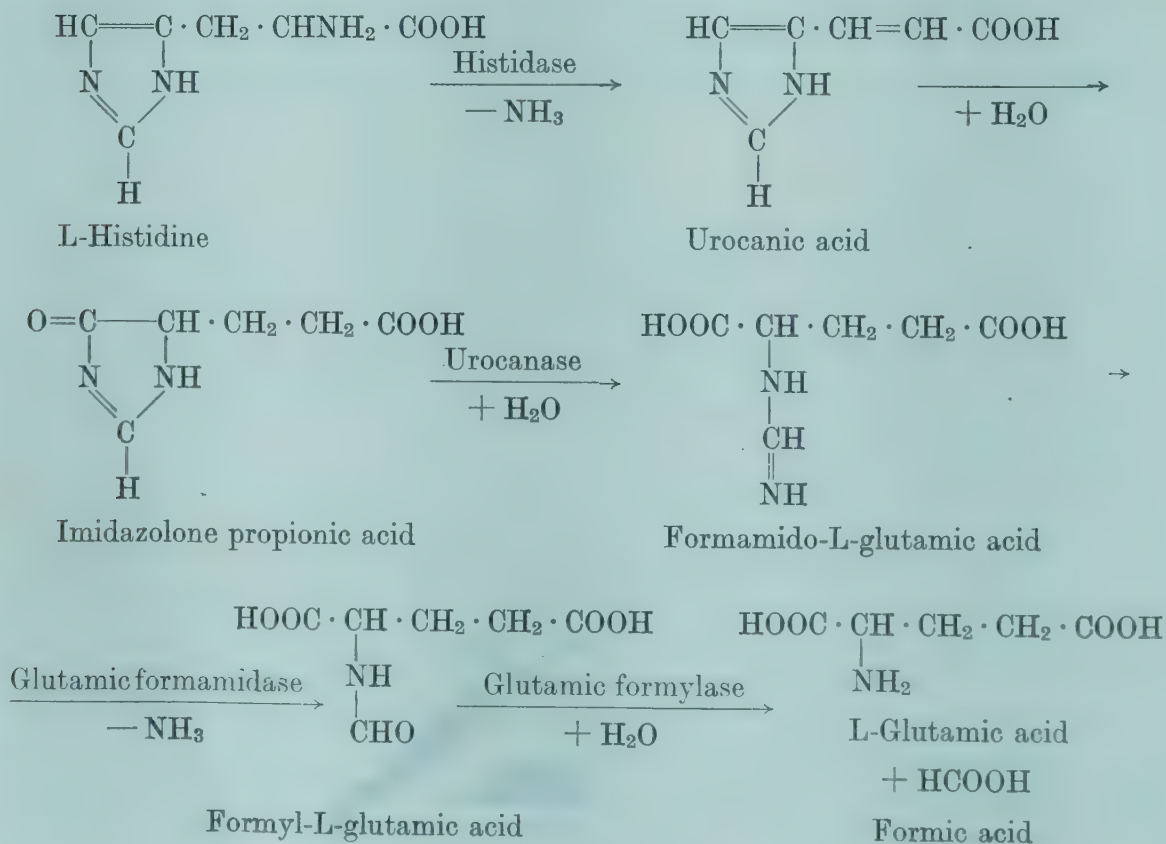
Eddlbacher used the term "histidase" for the entire enzyme system of the liver responsible for the breakdown of histidine; today, the term means only that enzyme which catalyzes the deamination of histidine to urocanic acid. A second enzyme known as urocanase [281, 287, 288] converts urocanic acid into an intermediate substance - probably formamido-L-glutamic acid -, which is then hydrolyzed by alkali to L-glutamic acid, formic acid and ammonia.



It is likely that water is added to urocanic acid before it is split, and the resulting product converted into imidazolone-propionic acid, which has not yet been isolated. Under the influence of urocanase, this substance takes up a second molecule of water to form formamido-L-glutamic acid [287, 288]. In liver homogenate experiments, the catabolic process usually stops at this point, perhaps because of the absence of a second enzyme system necessary to take up the C_1 -body (formic acid).

Extracts of *Pseudomonas* break down formamido-L-glutamic acid stoichiometrically into L-glutamic acid, formic acid, and ammonia. Two enzymes are involved in this phase. The first, glutamic-formamidase, converts the formamido-L-glutamic acid into formyl-L-glutamic acid and ammonia. The second, glutamic-formylase, hydrolyzes the formyl-L-glutamic acid to L-glutamic acid and formic acid.

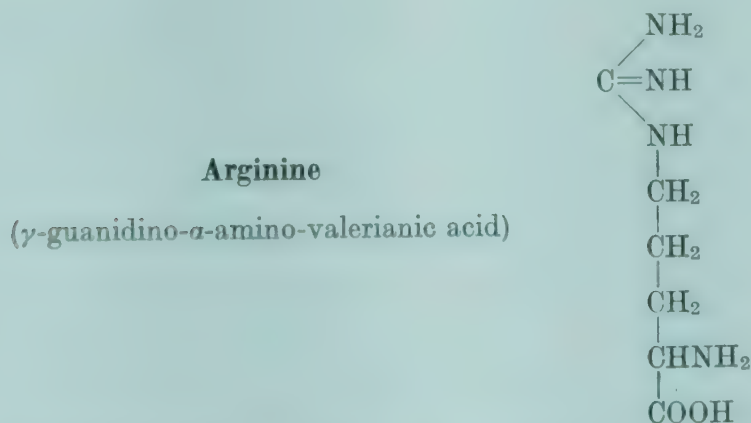
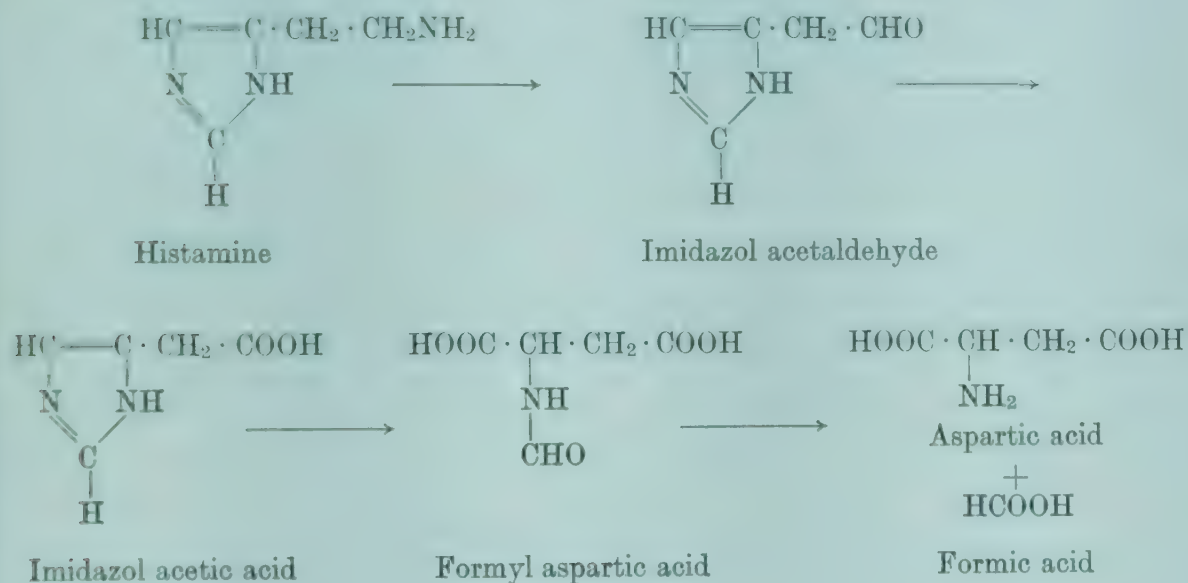
The following schema shows the degradation of histidine on the assumption that the animal body behaves in similar fashion. In favor of this schema is the observation that rats whose diet lacks folic acid excrete formamido-L-glutamic acid in the urine.



Formic acid is bound to folic acid, and can be supplied by the resulting compound in the processes of C_1 metabolism for the synthesis of serine, purines, and other C_1 -acceptors.

Histidine can be converted, by deamination and transamination into imidazol-pyruvic acid, which can then be reduced to imidazol-lactic acid.

Finally, histidine can be decarboxylated to *histamine*. The enzyme involved is present in animal, plant and bacterial cells. Histaminase (which is perhaps identical with diaminoxidase) breaks down histamine, probably via imidazol-acetaldehyde, to imidazol acetic acid. This can be oxidized to formyl-aspartic acid and ammonia. The former, finally, is hydrolyzed to formic acid and aspartic acid [289].



The importance of arginine in the formation of urea has already been discussed.

Arginine must be present in the diet of young rats for optimal growth. Rats can synthesize some arginine, but not rapidly enough for growth requirements. Arginine is necessary for the synthesis of tissue proteins (it is present in all of them), and for the synthesis of creatine and, eventually, the other guanidine derivatives. When the diet contains too little arginine, the various needs for arginine add up; in this case, e. g., young chicks grow poorly and have poor formation of feathers. When creatine is added to the diet, they grow better and the feathers develop normally, for the available arginine need be used only for the protein synthesis [290] (not to make creatine).

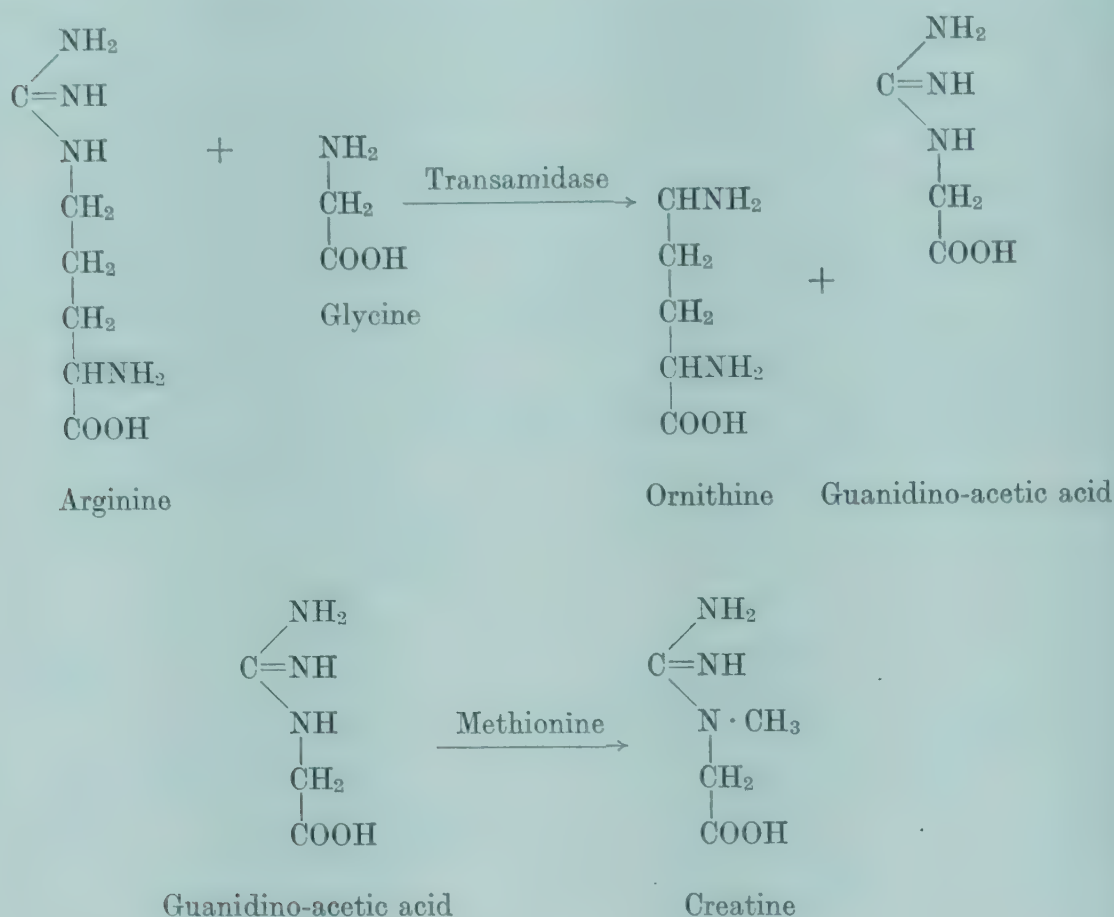
Full grown animals can maintain their equilibrium without arginine when their other amino acid needs are covered.

Arginine provides the amidine group for the *synthesis of creatine*. In invertebrate animals arginine itself takes over the function which creatine performs in vertebrates.

The guanidine group of creatine comes in part from arginine, as shown by Borsook and Dubnoff. They fed various animals arginine whose guanidine group was labelled with deuterium, and isolated from the urine deuterium-containing creatinine [291]. Experiments with argininic acid produced by deamination of arginine with nitrous acid, gave the same results [292].

The carbon skeleton and one nitrogen atom of creatine are derived from glycine.

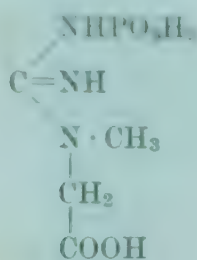
The kidney contains an enzyme, transamidase, which transfers the amidine group of arginine to glycine, converting the latter to guanidino-acetic acid. The latter is then methylated by methionine to form creatine. The arginine itself is converted to ornithine.



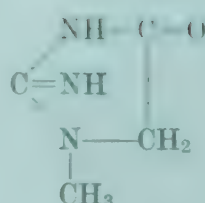
The amidine group can also be transferred to sarcosine, with the direct formation of creatine.

Creatine is present in all tissues, partly as phosphocreatine, a form in which high-energy phosphate is stored for liberation when needed to manufacture other high-energy phosphate compounds – e. g., to convert ADP into ATP (pp. 93, 94).

Creatine is normally excreted in the urine only before puberty and after the menopause. In all other cases, as a rule, it is first changed into *creatinine* before excretion by removal of a molecule of water.



Phosphocreatine

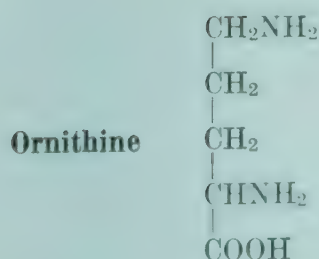
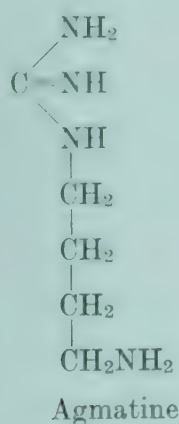


Creatinine

In certain muscle diseases (e. g., pseudohypertrophic muscular dystrophy) there is an increase in the urinary output of creatine. Apparently, the creatine cannot fulfill its function in the muscles and is thus not retained in the body. In such disorders, various substances can be tested for their ability to form creatine.

If arginine could be deaminated to the α -keto acid, further degradation of this keto acid (decarboxylation and β -oxidation of the guanidino-butyric acid) could lead directly to the formation of guanidino-acetic acid from the arginine, and this could then undergo methylation to creatine.

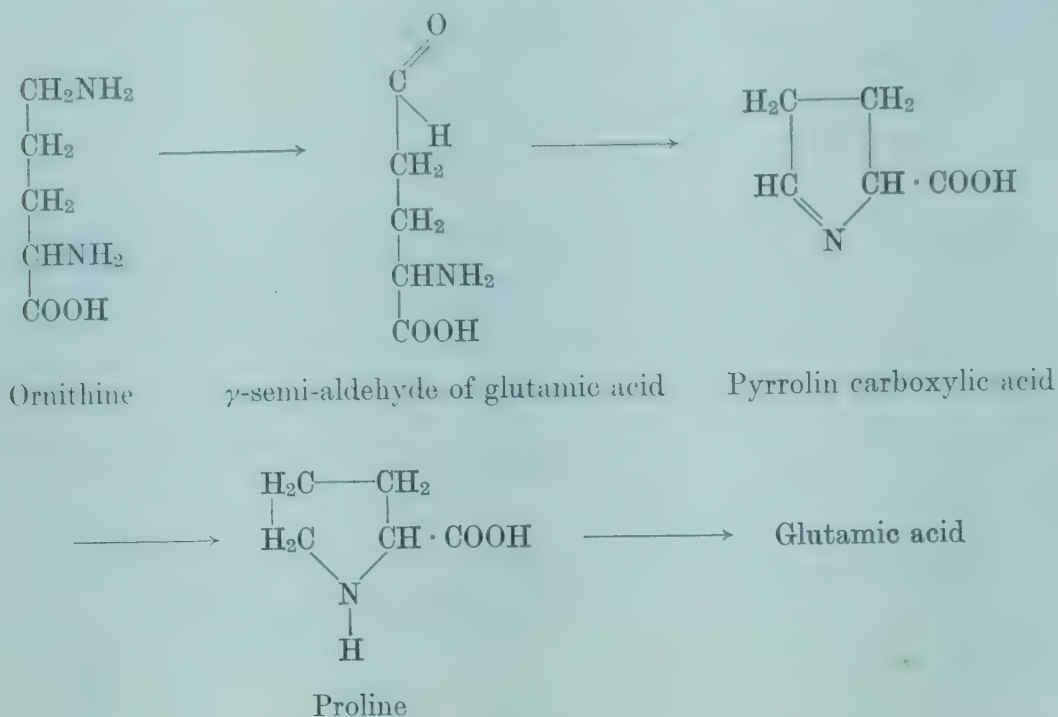
Arginine can be decarboxylated to agmatine. This base has been actually discovered in animal tissue (herring testis), but it is doubtful if animal tissues have the enzyme necessary for the decarboxylation of arginine. Diaminoxidase oxidizes agmatine to guanidino-butyric acid.



Ornithine is not a building stone for the synthesis of proteins, but occurs regularly in the synthesis of creatine and urea. Most of it may again be converted back to arginine. In the diabetic, 3 of the 5 carbon atoms of ornithine may be converted to glucose. Birds utilize ornithine in the detoxification of benzoic acid, forming ornithuric acid.

Ornithine labelled with stable, bound deuterium is converted by mice into proline and glutamic acid [293, 294], with the formation of the γ -semi-aldehyde of glutamic

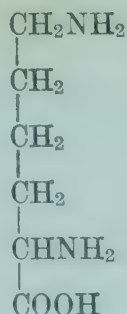
acid [295]. This closes its ring to form pyrroline-carboxylic acid, which finally, is reduced to proline. Proline can then form glutamic acid. Possibly, the γ semi-aldehyde of glutamic acid can be directly oxidized to glutamic acid.



By means of glutamic acid, thus, ornithine too joins the citric acid cycle and can in this way transfer 3 of its carbon atoms into glucose.

In cystinuria, there is sometimes excretion of putrescine, the decarboxylation product of ornithine. It derives probably from the putrefaction which occurs in the bowel (p. 373).

Lysine
(α - ϵ -diamino-caproic acid)



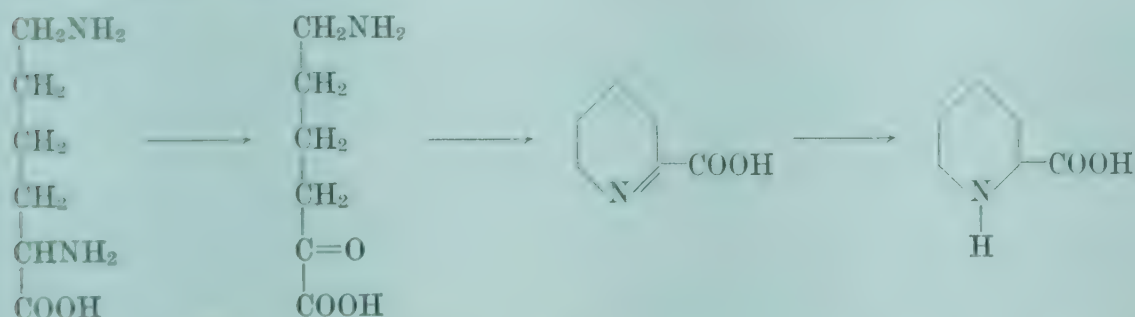
L-lysine is an essential amino acid for all animals investigated to date, and cannot be replaced by D-lysine. It does not exchange its α -nitrogen with other amino acids. In metabolism, it is relatively inert. It takes a long time for its nitrogen to be excreted as urea. Probably, it is an essential amino acid only because it is used in the synthesis of various proteins.

When an acetyl group, a methyl group, or another radical is substituted at the ϵ -amino group, the α -amino group can undergo deamination or transamination [296]. Conversely, the amino group of glutamic acid can be transferred to the corresponding α -keto-derivative of the ϵ -substituted lysine [297].

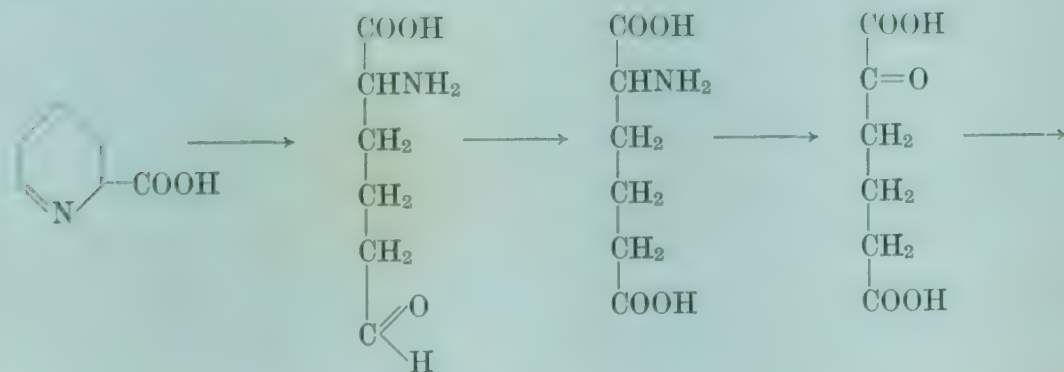
Liver homogenates can convert very small amounts of lysine by deamination of the ϵ -amino-group, into α -amino-adipic acid, thus breaking it down by way of α -keto-

adipic acid to glutamic acid, in this way, the nitrogen and carbon of this inert amino acid can be brought into the metabolic processes [298-300].

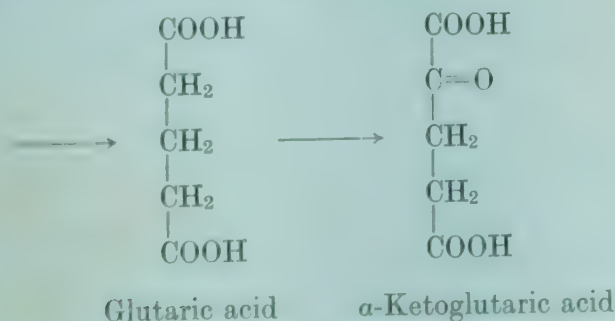
More recent studies in plants and in rat liver have shown that the α -amino-adipic acid is the result, not of ϵ -deamination, but of a cyclic intermediate product, pipercolic acid [301, 302]. When lysine whose ϵ -location is labelled with C^{14} or N^{15} is fed to rats, both isotopes appear in pipercolic acid. N^{15} at the α -amino group, in contrast, does not appear in any intermediate product or in any other amino acid. It must therefore be split off and excreted in the urine as urea. Probably, it is liberated by oxidation and α -keto- ϵ -amino-caproic acid is formed, which then is converted into pipercolic acid. In *neurospora*, lysine is oxidized by L-amino-acid oxidase to α -keto- ϵ -amino-caproic acid. The following scheme of the breakdown of lysine has been proposed by Rothstein and Miller [303-305]:



Lysine α -Keto- ϵ -amino-caproic acid 1¹-Dehydropipercolic acid Pipercolic acid



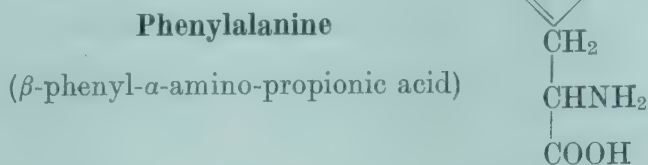
1¹-Dehydropipercolic acid ϵ -semi-aldehyde of α -amino-adipic acid α -Aminoadipic acid α -Ketoadipic acid



The process is not reversible in the animal body, and the intermediate products cannot replace lysine in the diet. Both α -amino-adipic acid and α -amino- ϵ -hydroxy-caproic acid act as antagonists to lysine and inhibit growth and the formation of hemoglobin [306].

Neurospora can synthesize lysine from α -amino-adipic acid, but not from pipercolic acid. This organism can also utilize D-lysine [307].

Escherichia coli forms lysine from α - ϵ -diamino-pimelic acid ($\text{HOOC}-\text{CHNH}_2-(\text{CH}_2)_3-\text{CHNH}_2-\text{COOH}$). This diamino-dicarboxylic acid is found in many bacteria [308-311]. It may be that bacteria can already begin the synthesis of lysine at the stage of aspartic acid [312].

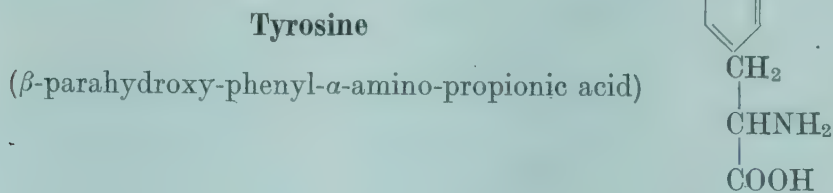


Phenylalanine is essential for most animal species, for they are apparently incapable of synthesizing the benzol ring. The first step in its catabolism is oxidation to tyrosine.

Phenylalanine was shown to be changed to tyrosine by Embden on the basis of experiments with artificially perfused liver [313]; but liver broth forms insufficient tyrosine from phenylalanine to show a positive Millon reaction [314]. Udenfriend and Mitoma studied this conversion [315]. Animal liver, and only liver, contains a hydroxylating enzyme system which specifically converts phenylalanine to tyrosine. At least 2 enzymes are present. Co-factors in the process are diphosphopyridine-dinucleotide, an aldehyde (not formaldehyde), and ferrous ions. In addition, oxygen is necessary [316]. The details of the process are not known.

One form of mental deficiency, known as *phenylpyruvic oligophrenia* (p. 404) shows an increase of phenylalanine in the blood and the excretion of phenylpyruvic acid, phenyl-lactic acid and phenylacetic acid in the urine [317, 318]. In this disorder, the hydroxylating enzyme system in the liver is lacking [319]. Its relationship to the function of the brain is not yet known.

If phenylalanine is not first converted to tyrosine, it cannot be broken down, but can only be deaminated to phenylpyruvic acid, which is either reduced to phenyl lactic acid, or oxidized to phenylacetic acid.



Tyrosine is not an essential amino acid, and can be replaced by phenylalanine. It belongs to the "ketoplasmic" amino acids and is the parent substance of melanin and the hormones of the thyroid and adrenal medulla.

The first step in the catabolism of tyrosine was determined in the study of *alkaptonuria*, a hereditary disturbance of metabolism in which there is excretion of homogentisic acid (hydro-quinone-acetic acid) in the urine. When such a urine is exposed to air, it becomes dark as the result of bacterial production of ammonia (alkaliniza-

tion. As a hydroquinone derivative, homogentisic acid is oxidized by the oxygen of the air to a brown quinone-type dye. When tyrosine or phenylalanine is fed to patients with alkaptonuria, the excretion of homogentisic acid increases.

O. Neubauer [320] proposed a schema for the route by which the 2 aromatic amino acids are converted to homogentisic acid; this schema is still accepted today. Phenylalanine is first oxidized to tyrosine, which is then deaminated to p-hydroxy-phenylpyruvic acid. The pyruvic acid residue can be easily decarboxylated and oxidized to an acetic acid residue. Normally, however, the side chain migrates before it becomes shortened. This occurs by the simultaneous introduction of a second hydroxyl group in a para position to the first. The migration is prerequisite to the opening of the benzol ring. If, for any reason, the side chain cannot migrate, then the p-hydroxy-phenylpyruvic acid (like phenylpyruvic acid itself) cannot be broken down further but can only be decarboxylated and oxidized to p-hydroxy-phenylacetic acid.

After the side chain has migrated and become shortened, the resulting homogentisic acid opens its ring and is converted to acetic acid and fumaric acid. This last reaction cannot be carried out by the alkaptonuric individual, who lacks the necessary enzymes.

Numerous experiments in the last 15 years on liver broth, liver slices, and liver homogenates, using labelled and non-labelled phenylalanine and tyrosine, have added various particulars to Neubauer's schema.

Liver preparations work differently on the 2 different optical forms. D-tyrosine undergoes rapid oxidative deamination through D-amino-acid oxidase with the formation of corresponding amounts of ammonia and urea. The oxidation of L-tyrosine proceeds more slowly, but little or none of the expected ammonia and urea can be demonstrated. Both isomeric forms are ultimately broken down to Millon-negative products [321-323].

In liver broth, L-tyrosine undergoes transamination with pyruvic acid and is thus deaminated. Thus, alanine can be regularly demonstrated in the degradation of tyrosine in liver broths; and the addition of pyruvic acid accelerates and enhances the reaction. Added α -keto-glutaric acid can readily accept the amino group and works even more rapidly than pyruvic acid; oxalacetic acid has approximately the same activity [324, 325]. The pyruvic acid used up is later replenished, probably from degradation products of tyrosine [324].

Tyrosine can also be deaminated by transamination in the kidneys. Here, glutamic acid takes up the amino group and is converted to glutamine [324]. Transamination of tyrosine must result in p-hydroxy-pyruvic acid. This substance is clearly demonstrated, however, only if there is a deficiency of ascorbic acid [324]: in the presence of the vitamin it promptly undergoes further degradation.

With the beginning of oxidation, the side chain migrates, and decarboxylation also begins. Probably this occurs as long as the pyruvic acid is still bound to the amino group of tyrosine.

P-hydroxy-phenylpyruvic acid itself is further broken down by liver preparations by way of homogentisic acid, but not as readily as tyrosine [322, 324]. Ascorbic acid is necessary for the introduction of the second hydroxyl group into the ring and for the shift of the side chain [325-327]. Its exact role, however, is not yet known. The second hydroxyl group is introduced by means of true oxidation, not by dehydrogenation [328].

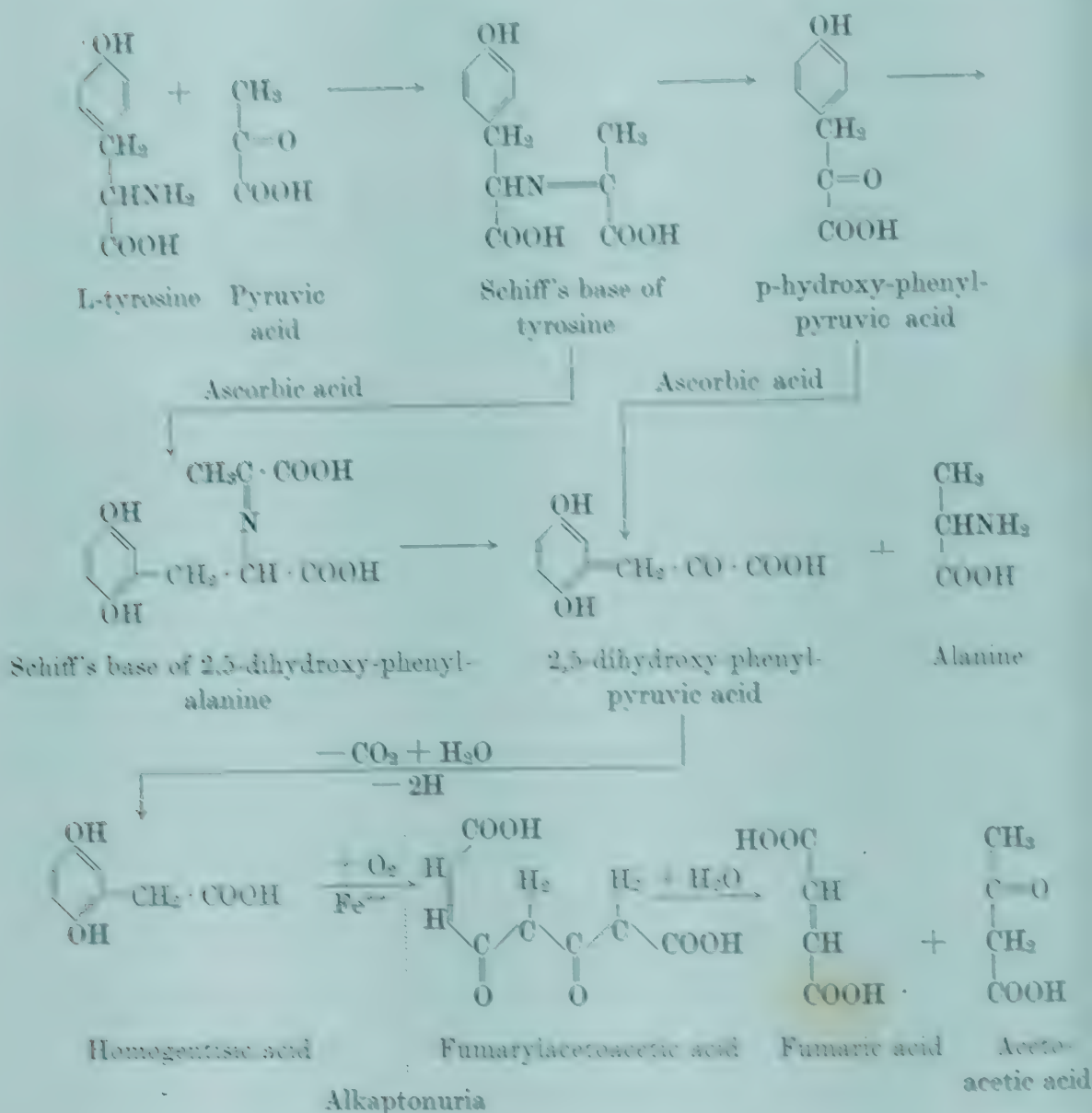
Certain liver preparations, e. g., those of rabbits, require additional ferrous ions for the oxidation of hydroxyphenyl-pyruvic acid [330].

When transamination is completed, there results 2,5-dihydroxy-phenylpyruvic acid or the Schiff's base of 2,5-dihydroxyphenylalanine. Both, however, are finally converted into homogentisic acid and this substance is oxidized to fumaryl-acetoacetic

acid as a result of opening of the ring, in which ascorbic acid is probably again involved. The fumarylacetoacetic acid is finally broken down into fumaric acid and acetoacetic acid. Both these end products connect tyrosine catabolism with the citric acid cycle. The acetoacetic acid is derived from the first and second carbon atoms of the side chain, and from the 3rd and 4th carbon atoms of the ring [329-332].

The degradation of tyrosine is depicted in the following schema.

Tyrosinosis



Tyrosinosis

In alkaptonuria, the breakdown of homogentisic acid is blocked. There is another disturbance, in which the degradation of tyrosine stops at the stage of p-hydroxy-phenylpyruvic acid: "tyrosinosis." This was first discovered by Medes in a patient who showed a further increase in the excretion of the keto acid when he was fed tyrosine [333]. In addition, this patient also excreted 3,4-dihydroxy-phenylalanine in small amounts (see section on the formation of epinephrine).

In 2 other cases, the spleen was removed [334]. This operation in itself has no influence on the breakdown of tyrosine; when, however, the liver is also diseased, it is incapable of breaking down tyrosine further than p-hydroxy-phenylpyruvic acid. The first patient, who showed spontaneous excretion of p-hydroxy-phenylpyruvic acid, suffered from cirrhosis of the liver; the other patient had hepatitis and first showed excretion of the acid when he was given tyrosine. The disturbance was thus latent in the 2nd patient. Ascorbic acid had no effect in either patient. In a 3rd patient without liver disease, splenectomy was necessary because of an accident; there was no demonstrable disturbance of tyrosine metabolism following the operation. Finally, a patient with hemolytic icterus was studied: she catabolized p-hydroxy-phenylpyruvic acid quite normally before splenectomy, but did not catabolize it further after the operation [336].

The p-hydroxy-phenylpyruvic acid is broken down only by the liver, to form acetoacetic acid, fumaric acid, and carbon dioxide. Whatever p-hydroxy-phenylpyruvic acid passes from the liver to the peripheral tissues can be metabolized only in the kidneys, where it is decarboxylated and oxidized to p-hydroxy-phenylacetic acid [324].

Liver function test using p-hydroxy-phenylpyruvic acid

Since p-hydroxy-phenylpyruvic acid is broken down only in the liver to form substances which no longer give the Millon reaction, it can be used as a *test substance* for liver function [335]. People with normal liver function break down up to 5 grams of the keto acid without difficulty. In the presence of liver disease, no more than 2 grams are broken down, and, according to the severity of the disorder, more or less of this is excreted.

In the test, the patient receives, on a fasting stomach in the morning, 2 grams of p-hydroxy-phenylpyruvic acid. The Millon reaction is used to test how much of the dose is excreted. If more than 8% is excreted (i. e., more than 160 mg), the liver is diseased [336]. Since normal urine gives a positive Millon reaction, the intensity of the reaction must be determined for 1 day before the test and 1 or 2 days after the test.

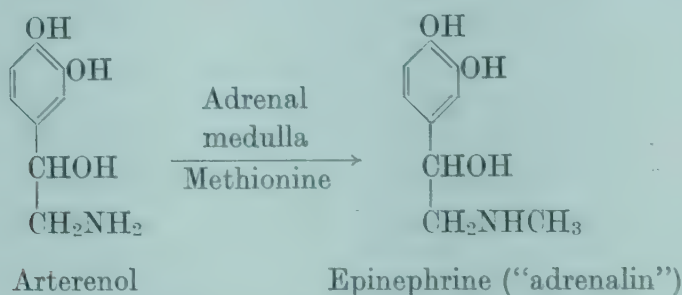
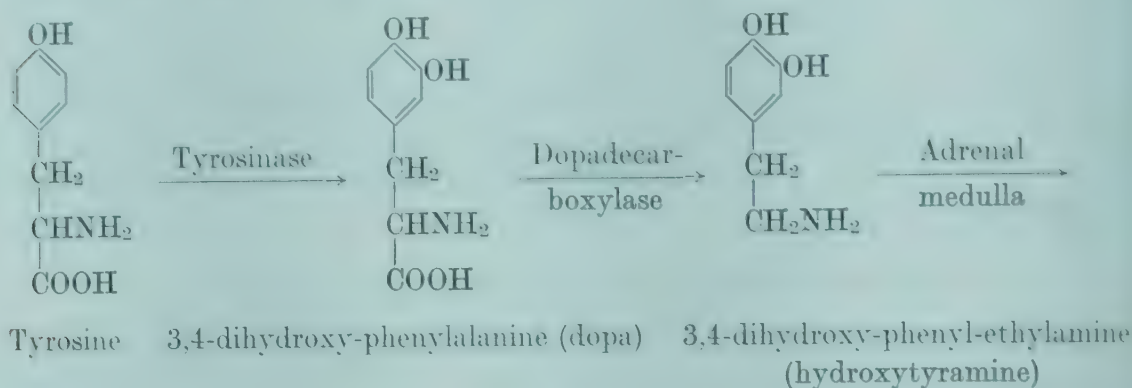
The test is very sensitive, and has been found useful in many clinics [337]. It has shown that the liver is involved in many more diseases than previously thought. Suppurative wounds, for example, damage the liver; the more severe the infection and the less the purulent discharge, the greater the damage to the liver. If the infection persists for too long a time, prolonged damage of the liver may occur. Too much thyroxine (thyrotoxicosis) also impairs liver function. Cardiac diseases also have an unfavorable effect. Of especial interest, however, is the relationship to the brain [338]. In many mental disorders, especially the endogenous, periodic, or episodic psychoses, the liver is as damaged as it is in severe cirrhosis. When the psychosis improves, the liver regains its normal function, as measured by the p-hydroxy-phenylpyruvic test.

In severe liver cirrhosis, the administered p-hydroxy-phenylpyruvic acid is split to phenol and pyruvic acid, and both substances appear in increased amounts in the urine [339].

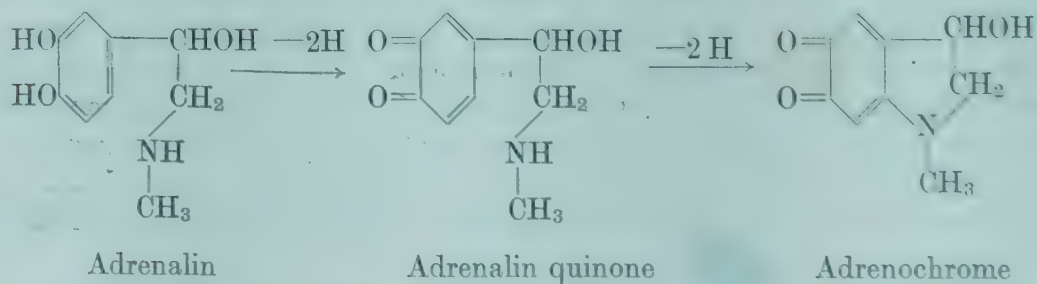
Formation of Epinephrine and Brown Pigments

The synthesis of epinephrine begins with the oxidation of phenylalanine to tyrosine in the liver. It has been shown that phenylalanine labelled with tritium (H^3) in the ring and with C^{14} in the carboxyl group is converted, by rats into H^3 -labelled epinephrine [340].

Tyrosine is first oxidized to 3,4-dihydroxy-phenylalanine ("dopa") by tyrosinase, a copper containing oxidase [341]; this was also found to be excreted in the first reported case of tyrosinosis described above (p. 396). Dopa is decarboxylated to 3,4-dihydroxyphenyl-ethylamine through the action of a specific "*dopa-decarboxylase*," which is found in various animal tissues (e. g., kidney), but not in the adrenal medulla. The next step occurs in the adrenal medulla: slices of this tissue produce epinephrine from this amine. It is not certain how and when the hydroxyl group is introduced at the β -carbon atom of the side chain. It is very likely that arterenol is first formed, which is ultimately methylated to epinephrine with the aid of methionine. The following schema gives the probable sequence of events:

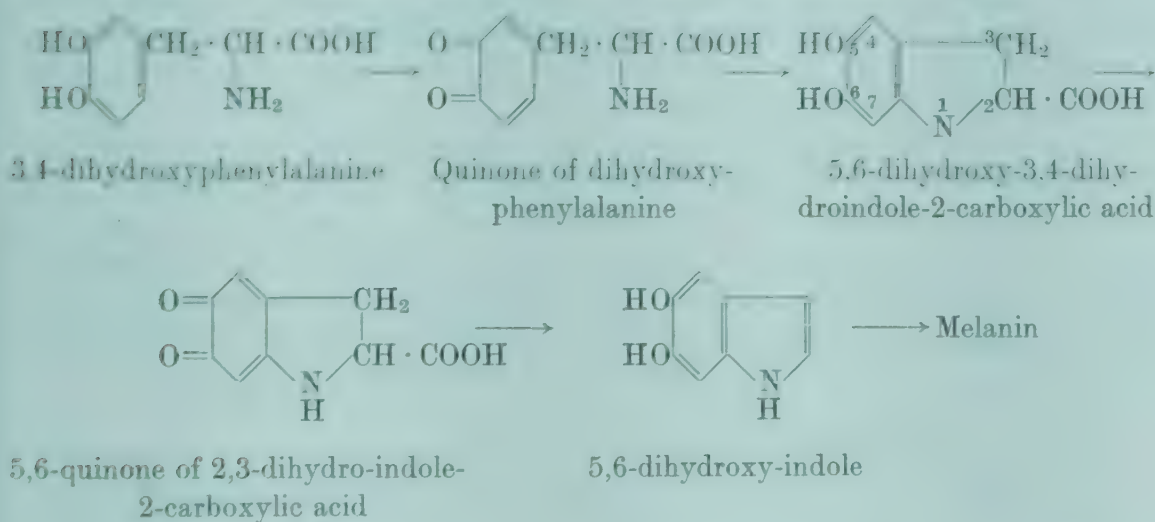


Epinephrine is dehydrogenated to *adrenochrome*, a red dye, by the action of cytochrome (perhaps also by tyrosinase) by way of the corresponding quinone. Adrenochrome can be converted to a dark pigment resembling melanin.

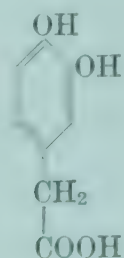


The skin of practically all animals, with the exception of albinos, contains the brown pigment *melanin*. Irradiation of the human skin with ultraviolet light causes increased formation of melanin in the skin. Melanin is found in malignant melanomas, and patients with this tumor excrete the substance in the urine. The squid secretes it as a protective screen against its enemies.

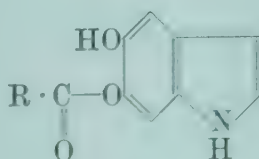
First, tyrosine is converted to 3,4-dihydroxyphenylalanine by tyrosinase. This is dehydrogenated to the 3,4-quinone of phenylalanine. Then, ring closure occurs between the amino group of the side chain and carbon 5 of the benzol ring, and at the same time both double-bonded oxygen atoms are reduced to hydroxyl groups. The result is 5,6-dihydroxy-2,3-dihydro-indole-2-carboxylic acid. This is again oxidized to form a red orthoquinone, the 5,6-quinone of 2,3-dihydro-indole-2-carboxylic acid (also called "hallachrome"). Decarboxylation results in the formation of 5,6-dihydroxy-indole [342]. From this compound, melanin ultimately is formed; the exact process is not known, but perhaps there is oxidation of 5,6-dihydroxy-indole to indole 5,6-quinone, and then polymerization of this latter compound [343].



In *melanuria*, which occurs in most cases of melanosis, melanogens appear in the urine which are converted to dark-brown pigments when the alkalized urine is exposed to air. One of these is homoprotocatechuic acid (pyro-catechol-acetic acid). Three chromogens, demonstrable by paper chromatography, are formed; these are derivatives of 5,6-dihydroxy-indole, and give a positive *Thormählen reaction* — i. e., they turn blue when sodium nitroprusside is added. In one of these compounds, one of the 2 hydroxyl groups becomes esterified, probably with a dipeptide of glutamine and glutamic acid [344].

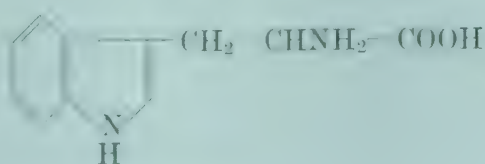


Homoprotocatechuic acid



Probable formula of the Thormählen substance

Tryptophane

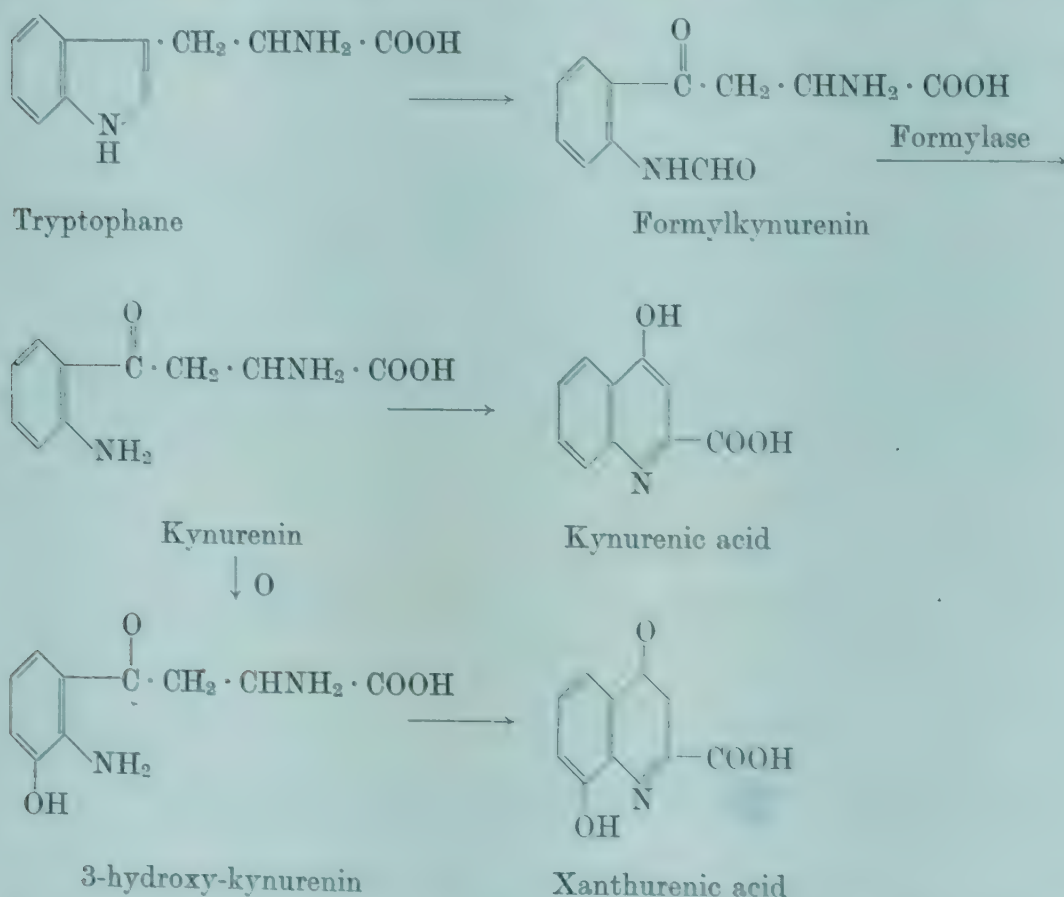
 (3-indole- α -amino propionic acid)


Tryptophane is an essential amino acid whose essential nature has been known longer than that of any other amino acid. Its reactions occur in all phases of metabolism. It can be catabolized in several ways.

Tryptophanase, a pyridoxal-phosphate enzyme, splits tryptophane to indole, pyruvic acid, and ammonia. In other manners of degradation, the indole ring also remains intact, for example when auxin (indoleacetic acid) or serotonin are formed.

Most of the tryptophane follows a metabolic pathway in which the indole ring is first affected, and which leads to the formation of kynurenic acid, which has been known to be the end product of tryptophane catabolism since Ellinger [345]. Xanthurenic acid is also formed in this process. It was Kotake who discovered the precise route which is taken by tryptophane in its degradation to these 2 quinoline derivatives [346, 347]. It is now known that this route is only one of many pathways which are possible following the splitting of the indole ring. Other pathways are the formation of nicotinic acid and of pigments.

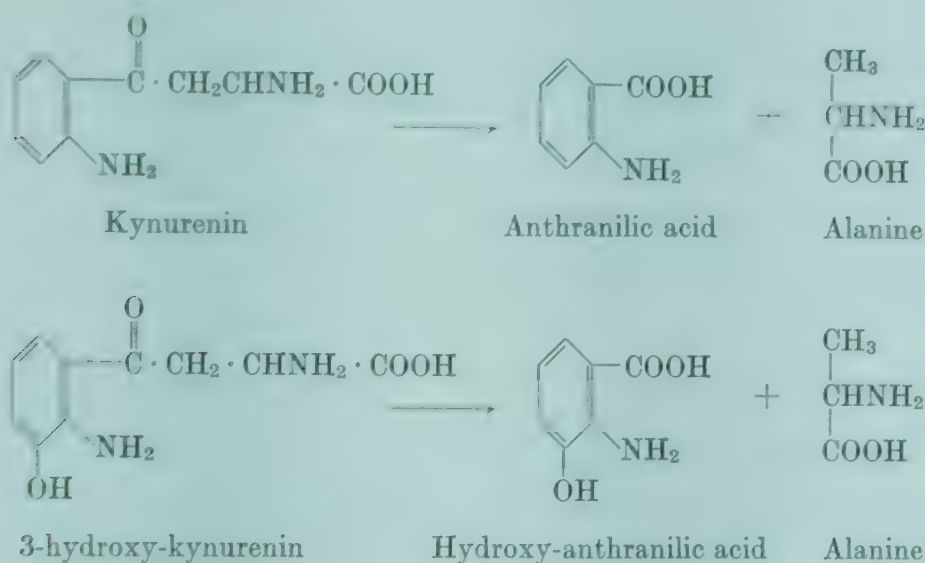
The first product which was demonstrated as a result of the action of enzyme preparations derived from guinea-pig liver on tryptophane is formyl-kynurenin [348]. An oxidation precedes this, the details of which are not known. Two oxygen atoms are introduced in this oxidation. It may be that an oxidase and a peroxidase work together. Formyl-kynurenin is hydrolyzed by a formylase to *kynurenin* and formic acid [348]. Kynurenin is yellow and gives the diazo reaction and may be the cause of the positive diazo reaction which occurs in the urine in various infections.



Kynurenin was first discovered by Kotake, but its composition was first established by Butenandt and his coworkers [349]. It can be further catabolized in various ways [350], for example by deamination of the α -amino-group, closing of the ring between

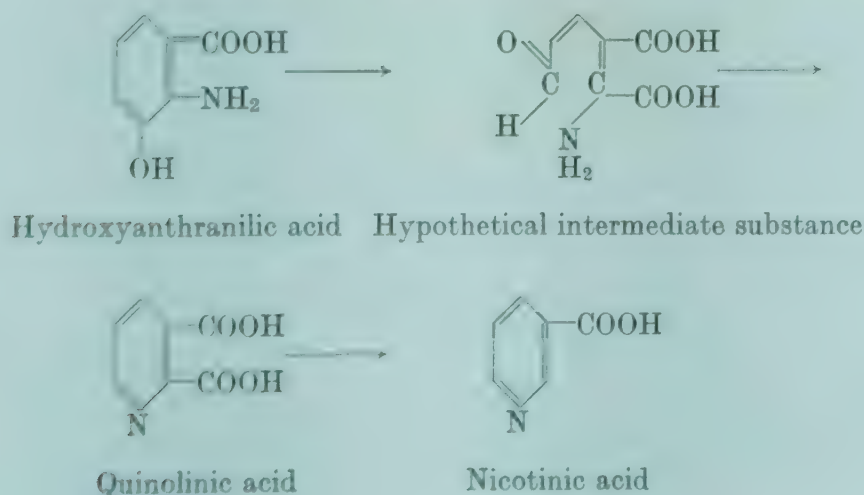
the α -carbon atom of the side chain and the amino group of the ring, and formation of *kynurenic acid* [351, 352]. By another route, kynurenin is first oxidized to 3-hydroxy-kynurenin and then xanthurenic acid is formed [353, 354]. In both quinoline derivatives, the nitrogen comes from the nitrogen of indole.

Kynurenin can also be hydrolyzed to anthranilic acid and alanine [353-356] with the help of the enzyme kynureninase, which requires pyridoxal-phosphate as coenzyme [357, 358]. In analogous fashion, 3-hydroxy-kynurenin is converted into hydroxy-anthranilic acid.



Experiments performed by Krehl and coworkers demonstrated the existence of a relationship between tryptophane and *nicotinic acid* [359]. Young rats nourished with a diet deficient in nicotinic acid amide showed normal growth when relatively large amounts of tryptophane were added to the diet.

These facts were verified by Heidelberger et al. They fed rats tryptophane labelled at various points and isolated correspondingly-labelled nicotinic acid in the urine [352, 360, 361]. The pathway proceeds by way of kynurenin and hydroxykynurenin, leading first to hydroxyanthranilic acid. The next intermediate product isolated is *quinolinic acid* [362], which is probably formed from hydroxyanthranilic acid: the carbon atom and attached hydroxyl group are oxidized to a carboxyl group, and the

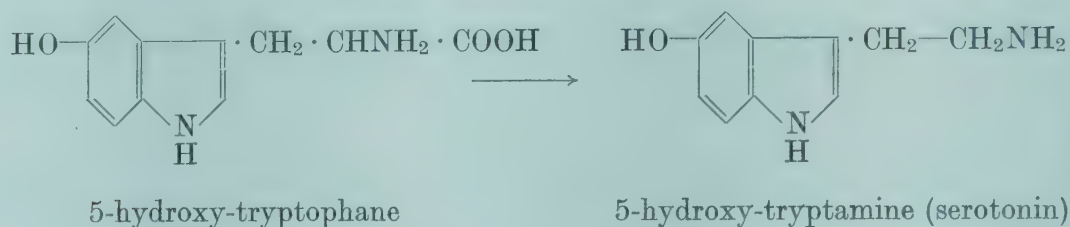


neighboring carbon is oxidized to an aldehyde group. The result is opening of the ring. A new ring then forms which includes the nitrogen of the amino group (p.109). Decarboxylation of the resulting compound results in the formation of nicotinic acid.

Kynurenin is also the parent substance of the yellow pigments of the eyes of the blow-fly and other insects [363].

Tryptophane is apparently not decarboxylated in the animal body unless it is first oxidized to 5-hydroxy-tryptophane. The product which then results is 5-hydroxy-tryptamine, also known as *serotonin*, which is present in serum and is produced in the cells at the base of the small intestinal crypts.

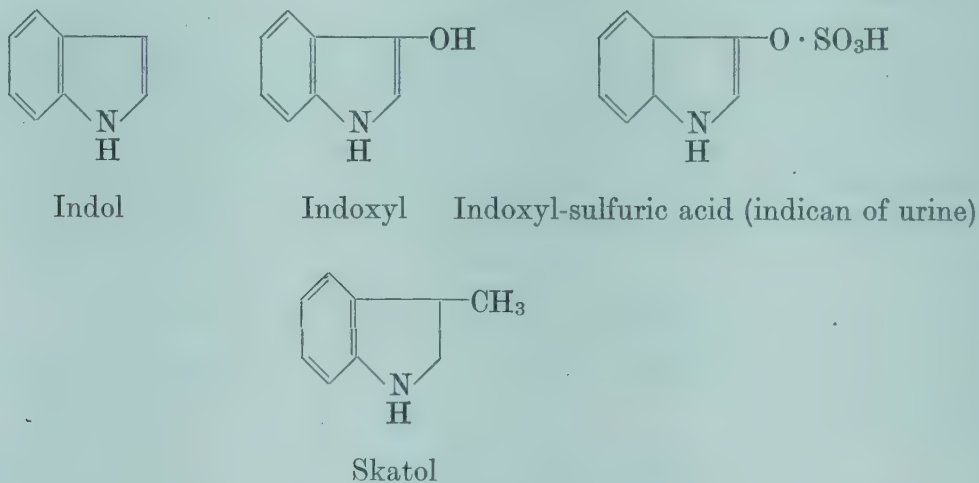
Tissue slices of guinea pigs and rats are capable of oxidizing tryptophane to 5-hydroxytryptophane. Various animal tissues contain a decarboxylase which is specific for this amino acid [364]. The product of methylation of serotonin (norbufotenin) is found in toad poison.



The *indole* of intestinal putrefaction, as already discussed, is formed by the action of the tryptophanase of coli bacteria which break tryptophane down into indole, pyruvic acid, and ammonia. Indole is probably oxidized in the wall of the intestine, or at the latest in the liver, to indoxyl and esterified with sulfuric acid. Indoxyl-sulfuric acid is the familiar *urinary indican*.

Indole is present in the serum and is also excreted in the urine. It is responsible for the aldehyde reaction which the urine of animals normally shows on warming.

Skatol likewise occurs in intestinal putrefaction in a manner as yet unexplained.



Excretion of Amino Acids in the Urine

As has been known for some time, small amounts of amino acids regularly appear in the urine. New, more sensitive tests have allowed more precise measurement of this loss of essential substances. Approximately 1.1 grams of free amino acids are excreted daily in the urine. This corresponds to approximately 180 mg. of nitrogen, or 1.2%

of the total urinary nitrogen. With this, approximately 2 grams of bound amino acids are also excreted, so that the total loss of nitrogen as a result of excretion of amino acids in the urine measures 500 mg per day.

The following values have been found for the daily excretion of free amino acids in the urine of the adult male (Table 68):

Table 68. Amino acids in the urine of normal adult men (mg/24 hours).
(After Harris [55])

	Range	Mean
Aspartic acid		<10
Asparagine	30-90	54
Glutamic Acid		<10
Glutamine		100
Glycine	70-200	132
Alanine	20-70	46
Amino-butyric acid		10
Valine		<10
Leucine	10-25	14
Isoleucine	10-30	18
Serine	25-75	43
Threonine	15-50	28
Cysteine and Cystine	10-20	10
Methionine		<10
Taurine	85-300	156
Proline		<10
Phenylalanine	10-30	18
Tyrosine	15-50	35
Tryptophane		
Histidine	110-320	216
1-methyl-histidine	50-210	180
3-methyl-histidine		50
Ornithine		<10
Lysine	10-50	19
Arginine		<10
Citrulline		<10
β -amino-iso-butyric acid and β -alanine	4-180	20

Two of the amino acids in the table, β -aminoisobutyric acid and 3-methyl histidine, have not yet been discussed.

According to Bickel, healthy children excrete fewer amino acids, namely, glycine, alanine, glutamine, glutamic acid, histidine, and, less frequently, serine and taurine [365].

The ratio of the amino acids in the urine to each other is different from their ratio in blood plasma. The clearance for the individual amino acids ranges between 0.5 and 2.5 c.c. per minute. For three of the amino acids, this value is greater: for glycine and histidine, between 5 and 10 c.c. per minute; for 1-methyl-histidine, 120 c.c. per minute, approximately corresponding to the rate of glomerular filtration.

When the urine is hydrolyzed in the process of determination of the bound amino acids, there appear glycine, glutamic acid, aspartic acid, methionine, valine, alanine,

and serine, together with small amounts of other amino acids (Table 69). Up to 70% of the glycine is derived from hippuric acid, of which approximately 1 to 2.5 grams is excreted daily in the urine. Glutamic acid is set free from glutamine and phenacetylglutamine, and aspartic acid from asparagine. The remaining amino acids must come from as yet unknown peptides.

The amounts of amino acids excreted in the urine vary from one person to another, even with the same diet. Perhaps these individual variations are determined in the genes.

In pregnancy, more amino acids are excreted. Threonine and histidine are especially increased. The latter reaches a maximum in the 4th month and then maintains this level until parturition. Threonine increases uniformly during pregnancy. During lactation, fewer amino acids are again excreted.

Many disorders have an effect on the mixture of free amino acids, both qualitatively and quantitatively. A disturbance in intermediate metabolism can result in an increase in the plasma concentration of one or several amino acids to such a degree that definite aminoaciduria results. To this "overflow" or "prerenal" aminoaciduria belong the increased excretion of phenylalanine in phenylpyruvic oligophrenia (p. 394) and the amino aciduria seen in a severe liver diseases.

In phenylketonuria, the level of phenylalanine in the plasma and in the cerebrospinal fluid rises to 15 to 60 mg^o. The concentration of the other amino acids, on the other hand, remains normal. The urine contains 100 to 300 mg daily.

Table 69. Peptide-bound amino acids in normal urine (mg/24 hours).
(After Mütting [366])

	20 males			20 females			values in the literature
	range	mean	calculated α -amino-N	range	mean	calculated α -amino-N	
Aspartic acid	85-202	141	14.80	92-207	149	15.64	192-251
Glutamic acid	180-561	313	29.73	217-614	428	40.66	470-640
Lysine	84-181	132	12.67	79-176	134	12.86	35-166
Arginine	21-49	31	2.54	20-50	31	2.54	20-94
Histidine	42-119	85	7.65	38-117	72	6.48	33-130
Tyrosine	12-26	17	1.31	13-28	24	1.85	15-57
Tryptophane	19-54	36	2.47	21-55	40	2.74	11-86
Phenylalanine	0-25	12	1.04	0-26	10	0.85	0-20
Hydroxyproline	0	0	—	0	0	—	—
Proline	36-107	80	9.76	28-94	65	7.80	67-94
Cystine	77-154	110	12.87	65-132	90	10.53	88-200
Methionine	217-510	365	34.31	198-482	338	31.77	247-494
Leucine	39-94	67	7.30	34-87	58	6.32	24-83
Isoleucine	7-26	18	1.96	6-29	21	2.29	0-26
Valine	98-172	144	17.28	82-158	144	13.68	22-40
Glycine	190-361	271	50.78	157-318	229	42.62	309-714
Alanine	115-198	162	25.43	121-201	178	27.95	81-142
Serine	97-177	139	18.07	105-212	170	22.61	70-206
Threonine	55-134	98	10.78	61-145	107	12.31	45-90
Taurine	0	0	—	0	0	—	0-13
Total		2221	260.75		2288	261.50	
Amino-N (according to Moore and Stein)			271.1			275.2	

Since it is the *liver* which is chiefly involved in the metabolism of amino acids, it is to be expected that more amino acids are excreted in liver disease. Actually a goodly portion of the liver must be damaged for this to occur, for this organ has a great functional reserve. The degree and the type of aminoaciduria vary from patient to patient and more or less parallel the severity of the illnesses. The most sensitive are cystine, β -amino-isobutyric acid, taurine, and colamine (colamine is actually not an amino acid at all, but is intimately involved in serine metabolism) (pp. 373, 375) [367].

Typical of the *renal forms* of aminoaciduria is cystinuria. In this condition, there is excretion not only of relatively large amounts of cystine, but also of arginine, ornithine, and lysine, and commonly also putrescine and cadaverine. Stein gives the following mean daily values: cystine, 0.74 grams; lysine, 1.8 grams; arginine, 0.83 grams; ornithine, 0.37 grams [368]. The other amino acids are not increased, and taurine may be somewhat diminished.

The current concept is that the increased excretion of cystine, lysine, and arginine is the result of impaired tubular reabsorption of these amino acids [369]. In favor of this concept is the fact that the plasma content of cystine and of the 2 basic amino acids is not increased, and in fact may be reduced [370-372]. In patients with cystinuria, the clearance of cystine is some 30 times normal.

The aminoaciduria which sometimes accompanies *Wilson's disease* is probably also renal in origin. In this condition there are degeneration of the caudate nucleus and of the lenticular nucleus, and the liver becomes cirrhotic. The copper content of many tissues is increased, probably including the cells of the renal tubules; the result is impaired reabsorption of the amino acids. The plasma concentration of the amino acids is normal.

When an individual lacks the enzymes necessary for conversion of *galactose* to glucose, so that he can no longer utilize galactose, he also excretes increased amounts of amino acids in the urine, specifically glycine, alanine, serine, and isoleucine. Here too the plasma levels of the amino acids are unchanged, so that the aminoaciduria is renal in origin. Hepatic cirrhosis also occurs. Children who have this disorder show slow mental development and develop a stare.

The *Fanconi syndrome* should be mentioned here. This is hereditary and is seen chiefly in children. There are a number of symptoms: rickets, acidosis, polyuria, renal glucosuria, hypophosphatemia, and aminoaciduria. Cystine is laid down in the tissues in large amounts. Glycine, alanine, serine, valine, leucine, isoleucine, glutamine, phenylalanine, lysine, arginine, proline, and cystine are excreted in increased amounts. Histidine, taurine, L-methyl-histidine, and β -amino-isobutyric acid remain unchanged. The amino acids are not increased in the plasma. In the other hand, the clearance of the amino acids which appear in the urine is greatly increased. This aminoaciduria, therefore, is also considered to be of renal origin. The reabsorption of glucose and phosphate is also impaired.

Review of Methods of Measuring Amino Acids

A few of the recently discovered methods for the determination of amino acids are also suitable for use in the clinical laboratory. Among these is chromatography utilizing columns or paper.

In *column chromatography*, it is best to use the ion-exchange resin Dowex 50 - 8, which is poured into tubes which are 0.9 cm. wide and 15 to 150 cm. long [373, 374].

The short tubes are used for the determination of basic amino acids; the long tubes, for neutral and acid amino acids. With a tube 150 cm. in length, one can separate all the amino acids in one procedure. The material being investigated is placed into the tubes: urine, protein-free filtrate of serum, deproteinized tissue extracts, or protein hydrolysates. As soon as it has dripped into the resin, buffers of various pH are placed into the tubes, and the outflow is collected fractionally in a series of test tubes. The amino acids in each test tube are measured quantitatively by the ninhydrin reaction [375]. Integration of the corresponding areas gives the amount of the individual amino acids. The exact method is given in the reports of Moore and Stein.

Paper chromatography [376–379] is simpler to carry out, but gives only qualitative or semiquantitative results. One or more drops of the material being studied are placed at the left lower corner of a piece of filter paper, and allowed to dry. The paper is then placed into an aqueous solution of an organic solvent (e. g., butanol-glacial acetic acid-water), and absorption is observed in descending or ascending direction. The amino acids are separated on the paper, each one concentrating at its characteristic location; staining with ninhydrin demonstrates these locations. One γ of glycine gives a clearly visible spot. When only one solvent is used, however, 2 amino acids may concentrate at the same spot. A different solvent (e. g., saturated phenol) is then set up in paper perpendicular to the first direction (two-dimensional chromatography). Fig. 72 is an example of such a 2-dimensional paper chromatogram of clupeine. The monographs of Cramer and Turba, and the original reports, give exact methods and results [378, 379].

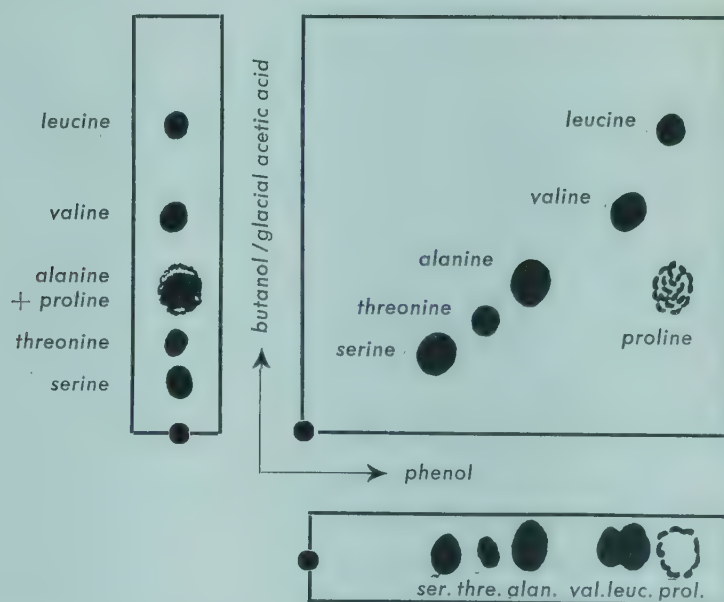


Fig. 72. The amino acids of clupein as separated by two-dimensional paper chromatography. The one-dimensional patterns in each solvent are also shown.

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CHAPTER VI.

Metabolism of Proteins and Amino Acids: Clinical Section

by Jan Waldenström

The Plasma Proteins

Methods

An account of the clinical manifestations and symptoms associated with disturbances in the level of the blood proteins should perhaps begin with a brief survey of the laboratory methods of greatest value to the clinician. The most fundamental analytic methods [1-6, 9-11] consist of fractionation of serum protein into albumin, α -, β -, and γ -globulins by means of Tiselius' *free electrophoresis* [1]; and the determination of the sedimentation constant of the protein fractions by means of the *ultracentrifuge* of Svedberg [2]. This latter method separates the serum proteins into 3 components, 4.5 S, 7 S, and 20 S, corresponding to molecular weights of approximately 70,000, 150,000, and 1 million, respectively. Both free electrophoresis and ultracentrifugation, however, can be carried out only in the well-equipped physico-chemical laboratory and can therefore be used only for special investigations. For clinical purposes, the original Tiselius method has been largely replaced in recent years by paper, agar and starch electrophoretic methods [3-6]. The results obtained by all these methods of fractionation will be discussed in considerable detail, as they provide the basis for the determination of serum proteins by their properties in an electrical field and by their molecular size. Recently, the method of immunoelectrophoresis has also gained considerable importance.

The clinician requires methods which are simple, inexpensive, and rapid. For these reasons, the blood erythrocyte sedimentation rate (E. S. R.) is of great value. Fåhræus in 1921 showed that it was a protein, fibrinogen, which was responsible for the marked acute increase of the sedimentation rate, in, e. g., pneumonia [12]. The chronically elevated sedimentation rate is almost always the result of an increase in γ -globulin.

There are many simple methods for the rapid determination of blood proteins. In 1936 Bing introduced the formol gel reaction, widely used in tropical medicine, into Europe [13]. He showed that a positive *formol gel reaction* (that is, rapid gelling of the plasma, but not of the serum, on addition of formalin) indicated elevation of the fibrinogen content of the plasma. Rapid gelling of serum, on the other hand, indicates increased globulin.

Most of the so called "lability tests" of serum are only qualitative measures of the serum proteins which detect both an increase of globulin and a decrease of albumin.

This is especially true of the *Takata-Ara reaction*, which is widely used in Europe, and *Hanger's cephalin flocculation test*, used throughout the United States. Originally, these were thought to be "liver function" tests, and their true nature as measures of the blood proteins is often forgotten. The most strongly positive Takata-Ara and Hanger tests are found in other disorders associated with hyperglobulinemia, such as multiple myeloma and lupus erythematosus disseminatus, and not in cirrhosis of the liver. Certain cases with reduced serum albumin, notably nephrosis, also show strongly positive Takata-Ara and Hanger tests.

The *thymol turbidity reaction* of MacLagan [14] is also usually considered to be a liver function test. It gives important information in hepatitis. In all probability, this test is positive because hepatitis is caused by a virus. In hepatitis, as in chronic liver disease ("cirrhosis") the antistreptolysin titer is often also nonspecifically elevated. Experiments have shown that the thymol turbidity reaction is often positive in other virus infections, such as measles and infectious mononucleosis. It is possible that the thymol turbidity test indicates the formation of anti-virus antibodies in the serum globulin. However, false positive thymol reactions may also occur, for the electrolyte content of the buffer system of the thymol reagent is very small, and the euglobulins present in the serum may precipitate, e. g. in macroglobulinemia. This precipitation has nothing to do with the thymol reaction and occurs even in the absence of thymol.

Kunkel devised his *zinc sulfate* test for the quantitative determination of gamma globulin [15] and Wuhrmann and Wunderly use a cadmium test to distinguish certain sera with increased β -globulin from those with high γ -globulin [16]. This cadmium test has been thoroughly investigated and found to be reliable [17]. It was interesting to note that the pathological β -globulins belong to two groups, one cadmium positive and the other negative. These methods have been more or less superseded (except for screening purposes) by paper electrophoresis, which provides a simple method for the direct separation of the different globulin fractions and their quantitative determination.

Van Slyke's *copper sulphate test* is a simple method for the quantitative determination of the total serum protein [19]. We have checked the method against the Kjeldahl values in a very large number of various hyperproteinemic sera and have found it to be quite reliable. For clinical purposes, therefore, as long as it is only the total serum protein that is determined, one can use Van Slyke's method instead of the more cumbersome Kjeldahl method.

Certain pathological globulins have the property of precipitating or gelifying in the cold, and are therefore called *cryoglobulins*. (See page 442.) Very rarely thermoglobulins (pyroglobulins) coagulating at 56° C. are present [19].

Hereditary Disturbances of Protein Metabolism

Clinical disturbances of protein metabolism may be divided into (1) congenital and hereditary types and (2) acquired. The acquired forms may be either secondary, symptomatic, or "idiopathic." The following section discusses the hereditary disturbances.

Garrod [20] has described disorders which are due to what he called an "*inborn error of metabolism*." These diseases, in which there is an interruption - i. e., block - of normal metabolism at a certain point, have contributed much to our understanding of metabolic processes. Conclusions derived from the study of such disorders have been verified by means of isotopes. New hereditary disorders of metabolism are con-

slowly being discovered, investigated, and placed in their proper genetic classification. In several it has also been possible to find effective methods of substitution.

Sometimes the exact nature and cause of a disturbance in metabolism has been determined, and often the cause is found to be an unpaired function of a given enzyme. There are also diseases which are due to inadequate formation of another specific protein (for example, hemophilia). Since enzymes are proteins, it is tempting to attribute the entire group of inborn errors of metabolism to primary disturbances of the synthesis of certain proteins. This concept is important because of our knowledge that genes have an important function in the formation of proteins. It is still a question whether, within the gene molecule, nucleic acid acts merely as a sort of matrix for the development of protein, or whether it always remains bound to protein. It is also likely that there is a separate gene for each enzyme. This concept was established by Beadle [21] in his brilliant experiments on the biochemistry of *Neurospora* mutants. Studies of the genetic control of metabolism have also provided basic knowledge of the nature of many clinical disorders. Human metabolism is governed by a genetically controlled enzyme pattern. Each inborn error of metabolism could thus be explained as due to the deformation of one specific protein template.

We shall not discuss here those disturbances in the metabolism of carbohydrate, fat, pigments, etc., which are due to congenitally defective enzyme systems. Figure 73 gives a schematic presentation of the mechanism of such hereditary metabolic disturbances in general. The influence of toxic factors is also important.

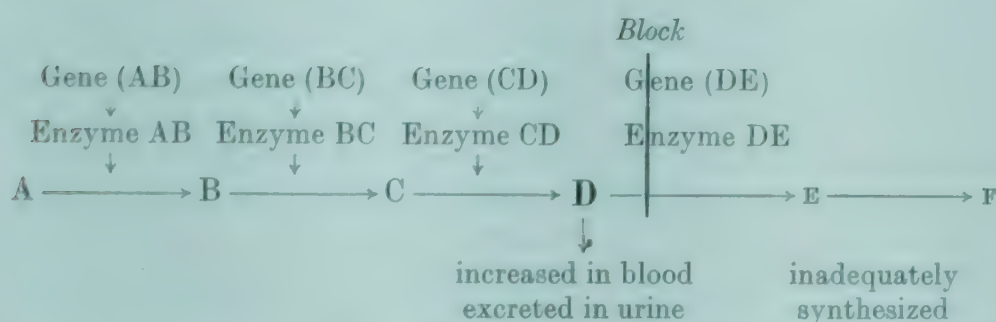


Fig. 73. Schema of a typical hereditary disturbance of metabolism. Damage to gene (DE) results in a defective enzyme DE, with the result that less E and F are formed and the amount of D in the body increases. Treatment consists of administration of E, which is then normally converted to F.

Coagulation defects

Hemophilia is without doubt the oldest and best known hereditary disease of the blood proteins. In this condition, there is inadequate production of a special globulin, antihemophilic globulin (AHG). The heredity of the disorder is that of a sex-linked trait. The theory of hemophilia has been complicated by the discovery of a similar disease with similar inheritance, but due to a different mechanism (lack of another plasma protein called plasma thromboplastin component or PTC). It is evident that there must be two different loci on the sex chromosome for the genes governing the synthesis of these two substances. It is very interesting that the severity of the disease also remains constant in the same family and is thus inherited (Koller [22]). It remains true to type. This must be an instance of multiple allelism where several mutations in

the same template lead to disturbances (deformations of protein molecule?) of different functional severity.

Quite recently a third hemophilia-like disorder has been extensively studied. This is obviously identical with the dominant autosomal condition which was previously thought to be a "thrombasthenia" and has been called v. Willebrand-Jürgens' disease. The episodes of severe bleeding are caused both by a low AHG as in true hemophilia and by a prolonged bleeding time. The term "vascular hemophilia" is quite misleading, however, as it has been shown in an extensive study on a large number of cases in Sweden that this bleeding is stopped by the administration of a protein substance separable from fibrinogen and AHG [23].

The complexity but also the simplicity of genetic causation are well seen in all these three "hemophilias." Each always *remains true to type* in the same family and is obviously a mechanism unto itself. Analysis of inheritance has done much to establish clear entities and thereby given the foundation for adequate substitution therapy.

Other hereditary disturbances of protein synthesis necessary for blood coagulation include *congenital hypofibrinogenemia* (recessive), *hypoprotrombinemia* (two types), and possibly other as yet incompletely investigated blood disorders.

Other defects of serum protein synthesis, etc.

Among the normal serum proteins, γ -globulins are of special importance because these comprise the fraction which contains antibodies. It must be emphasized that one should never use the word " γ -globulin" but only speak about the " γ -fraction" – that is, the "family" of various proteins behaving like γ -globulins in the electrical field. It would seem probable that this large group of proteins would be genetically non-homogenous. This, however, is not so. A well defined congenital disorder occurs in the genetically determined absence of the γ -fraction in the blood. Recent studies have shown that this condition is probably sex-linked, for it has thus far been reported with certainty only in male children [24]. Certain problems of protein chemistry in this disorder will be discussed later. The acquired type, of course, occurs in both sexes.

Wollheim has recently published some observations on cases of typical so-called acquired hypogammaglobulinemia in females. He was able to trace two of these patients back to common parents, who were born in 1790. It is therefore probable that a recessive trait may also be responsible for this type of gamma-globulin disturbance.

The clinical picture of agammaglobulinemia is of great theoretical and practical interest. It was originally described by Bruton in the United States [25, 26] and has since been reported from other countries. The patients are children, whose chief complaint is a great susceptibility to bacterial infections. The formation of antibodies is impaired, so that the patient often has, in succession and repeatedly, pneumonia, mumps, meningitis, etc. Before the era of antibiotics, such children died early in life. Now, however, their life is prolonged by means of antibiotics. Human γ -globulin is of definite value but the menace of bronchiectasis is still present. In Sweden the disorder was first observed in two brothers [24]; a third case in Sweden was traced to intermarriage of first cousins. Later observations have confirmed the sex-linked inheritance assumed by us [27, 28]. In Sweden one maternal cousin of the two brothers has also been found to suffer from the disease (Kulneff, unpublished). Good and Varco have studied skin transplantation in agammaglobulinemics [29].

Electrophoresis of the serum or plasma in such cases shows either marked reduction or nearly complete absence of the gamma component; hence the terms *hypogammaglobulinemia* or *agammaglobulinemia*. Pedersen showed on ultracentrifugation that the 7 S component was absent [24]. By serologic methods, a small amount of γ -globulin can always be demonstrated, and it is possible that total absence of the γ -fraction is incompatible with life. It is not yet clear whether the disorder is a result of lack of plasma cells (hence a "morphologic" malformation) or a functional weakness of a common template necessary for the formation of γ -globulin. Further studies may be expected to shed light on the problem of plasma cells and antibody formation. There is also an acquired form of agammaglobulinemia which occurs later in life and affects both men and women. Low γ -globulin values may also be found in lymphatic leukemia and myeloma as a symptomatic disturbance [16].

Immunoelectrophoresis has demonstrated absence of γ itself and of γ_{1A} and γ_{1M} components.

In 1954, Bennhold described *absence of serum albumin* in a healthy woman and her brother [30, 88] (see page 426). Study of a number of specific protein substances which are found in the albumin fraction would be of importance in such patients. Another interesting abnormality of serum protein that is inherited as a dominant character is the double albumin described by Knedel [31], which does not seem to be too rare in Germany (Knedel, personal communication).

With the exception of antihemophilic globulin, AHG, which itself may act more or less like an enzyme, all the diseases which have been discussed up to now are considered not as disturbances of enzyme synthesis but as interference with the synthesis of serum protein. Japanese investigators, however, have described a syndrome which they explain as the result of impaired catalase function of the blood [32]. A patient with necrotic ulcerations of the mouth was observed in whom rinsing the mouth with hydrogen peroxide failed to cause foaming: the blood of this patient was found to lack catalase. Other members of the patient's family also showed oral ulcers and diminished blood catalase. The disorder was named "*acatalasemia*." It is probably inherited as a recessive characteristic, and has thus far been described only in Japan. A large number of other enzyme defects have now also been localized to the erythrocytes; e. g. in hypophosphatasia, galactosemia, and a number of hemolytic conditions (acholuric jaundice, anemia due to primaquine [33], sulpha drugs, favism [34, 35]). The hemolysis often found in Negro patients after primaquine develops only in those hereditarily predisposed, where the function of an enzyme is defective. Other such drug sensitivities on the basis of an inherited enzyme defect are seen with succinyl choline in pseudocholinesterase defects, and also the acute attack in acute porphyria, an inborn error of metabolism, characterized by sensitivity to barbiturates.

A second disease showing absence of a specific enzyme in the blood is one type of *idiopathic methemoglobinemia*: in this disorder, there is lack of formation of a reductase, an enzyme which is necessary for the normal reduction of methemoglobin to hemoglobin [36].

Bennhold was the first to recognize and study the *transport function* of blood proteins [37]. Since his first reports, a series of specific binding-proteins have been found. The largest amount of work has been done on the globulins which bind lipids, iron, copper, and zinc. Haptoglobin, which binds hemoglobin, is discussed below (see vol. 2). One copper containing protein is called ceruloplasmin. Recent work has shown that this protein is markedly decreased in *Wilson's hepatolenticular degeneration* [38]. Certainly, there are other disturbances in this disease - for example, alterations

in renal tubular function – but it seems likely that the fundamental abnormality may be a deranged copper metabolism possibly because of impaired synthesis of ceruloplasmin. Deposition of copper in the liver could explain the development of cirrhosis, and the damage to the basal ganglia has similarly been attributed to the toxic effects of copper. The actual situation is probably quite complicated, for ceruloplasmin binds copper in a very firm manner. Loosely-bound recently absorbed copper is found in the albumin fraction (page 420). An explanation for the positive copper balance must be found. Hereditary *lack* of transferrin has not been observed, perhaps because it is a lethal factor.

Pauling has recently introduced the term “*molecular diseases*” to explain a new concept of illness [39] where the globin in hemoglobin is malformed for genetic reasons. The diseases which we have discussed to this point may also be considered molecular diseases. Pauling’s concept of the term, however, postulates that small chemical changes in the protein molecule may result in disease states. As in so many other investigations of protein substances, it was the method of electrophoresis which clarified the problems, and especially the new method of paper electrophoresis has been valuable for clinical purposes. Pauling and his associates investigated the electrophoretic behavior of hemoglobin in various diseases. They found that the hemoglobin S in cases of sickle cell anemia has a different electrophoretic pattern from that of normal hemoglobin A and can be readily separated from the latter. Furthermore, the reduced form of this sickle-cell hemoglobin S was found to have a different molecular configuration from that of normal hemoglobin. The development of sickled red cells under conditions of reduced oxygen tension depends on this change in the molecular structure of reduced hemoglobin S in saturated solution. The porphyrin portion of the hemoglobin molecule remains normal; hence sickle cell anemia is due to a disturbance in the development of the protein portion of the hemoglobin – that is, the globin.

Previous genetic studies had already established that sickle cell anemia occurs only in homozygous individuals [40]. The corresponding heterozygote, when studied by special laboratory technics, shows only a tendency to form sickle cells. By paper electrophoresis, it has been shown that the heterozygote has some 30% of sickle-cell hemoglobin in his erythrocytes, while in the homozygote 80 to 90% of the hemoglobin is abnormal. The other 10–20% is fetal hemoglobin F. This suppression of hemoglobin F formation, when A synthesis is speeded up as in the normal and the reversal to F when A is not properly formed is an important illustration of the interaction of genes. All these findings are of the greatest importance, for they show the manner in which homozygosity and heterozygosity are quantitatively expressed in such chemical errors of metabolism of protein synthesis.

Since this work many other abnormalities of globin development have been discovered and correlated with specific hereditary blood disorders.

It is perhaps not so well known that one special type of hereditary methemoglobinemia first described by Hörlein [41] is also regarded as due to a disturbed synthesis of globin (Hgb M).

Our knowledge regarding malformation of protein has been greatly advanced by Ingram’s [42] recent studies. By disintegration of the pathological globulins, this author showed that two of the resulting peptides were different with respect to a single amino acid in hemoglobin S and A. This difference explains their different patterns of migration. Such “fingerprints” of malformed protein molecules will be of fundamental value for the exact analysis of inborn errors of metabolism in general.

Transport Function of Blood Proteins

Bennhold was the first to describe the principles of the *transport function* of blood proteins [37, 43]. Many of the smaller molecules in the blood would simply pass directly from the blood plasma into the renal glomerular filtrate if they were not bound to a large molecule. Such binding may be comparable to mere adsorption or it may be a type of binding in which a special protein group binds a specific material (e. g., transferrin binds iron). This type of "vehicle function" was first demonstrated by Bennhold by means of dyes, but he anticipated that it would hold also for natural body substances. We shall discuss this question later; here we mention a number of amino and other substances, whose transport in the blood depends on their binding to a blood protein: iron (the protein is transferrin); copper (albumin-ceruloplasmin system); calcium (albumin); zinc (β -globulin); thyroxin (α -globulin); fats (α - and β -globulins); bilirubin (albumin); sulfonamides, penicillin, polysaccharides, et al.

Among the transport proteins, *transferrin* or siderophilin is of especial interest. Holmberg and Laurell showed that when iron is added to serum it combines with serum protein up to a maximum level of approximately 300-350 μ of iron per 100 cc of serum [44]. With the addition of iron, the color of the serum changes to yellow-red. The same phenomenon was found by Schade and Caroline [45] in studies of so-called "conalbumin" of egg protein. The iron-binding protein travels with the β -globulins on electrophoresis, and its molecular weight is approximately 90,000. The combination of iron plus this protein can be crystallized out [46], and Koechlin was able to crystallize the protein itself without attached iron [47]. The ability to bind iron is strongly influenced by the pH. Transferrin is specific for iron. The upper limit of normal serum transferrin content corresponds to an iron-binding capacity of about 350 μ of iron. The concentration of transferrin in the serum is always expressed in this way; i. e., as the total iron-binding capacity of the serum (T. I. B. C.).

Wallenius studied the distribution of plasma iron in the various serum proteins by means of radioactive iron [48]. Transferrin (i. e., the specific β -globulin) was apparently the only transport form of serum iron. Ferritin does not occur in normal plasma but is present in large quantities in severe liver damage [49]. The actual determination of plasma iron will not be discussed here (see vol. 2, Pollycove, and the review by Laurell [50]). We shall merely mention that the saturation of transferrin varies markedly in different disorders. Normally, about one-third of the transferrin is saturated with iron. In therapy, therefore, a large amount of extra iron can be bound in the blood. In hemochromatosis, on the contrary, almost all the transferrin is saturated with iron, and even a small additional amount cannot be further bound to protein. If iron is given intravenously to such patients, toxic symptoms rapidly ensue. The total amount of transferrin is increased in only 2 clinical conditions: in the last months of pregnancy (450 μ of iron) and in chronic iron deficiency. In chronic iron deficiency, the serum iron level is so low that only $1/10$ to $1/20$ of the transferrin is saturated with iron. When there is increased utilization of iron for new blood formation, for example in pernicious anemia during beginning remission, the serum iron falls to low values. When blood formation is poor, as in untreated pernicious anemia, the serum iron is often elevated, but falls rapidly, e. g., when high fever occurs. The total amount of transferrin remains normal in pernicious anemia. It is thus clear that the utilization of iron in the bone marrow and in other tissues (reticuloendothelial system) regulates the serum iron but does not usually affect the serum transferrin. In one illness both the serum iron and the serum transferrin are elevated: this occurs in

acute hepatitis in the 2nd to the 5th week. The explanation for this observation is unknown, but it is obvious that the liver is involved in iron metabolism. The normal serum iron fluctuates markedly from day to day [51] and its regulation is obviously not very rigid.

Absorbed copper is initially bound to albumin but with the formation of ceruloplasmin rapidly passes into the α_2 -fraction. The synthesis of ceruloplasmin is probably interfered with in Wilson's disease and radioactive copper remains for an abnormally long time in the albumin fraction [52, 53].

Calcium is present in the plasma in two forms: ionized and nonionized. The latter form is chiefly bound to the carboxyl groups of various proteins. Albumin has the highest binding capacity for calcium; β -globulin binds little calcium. (See vol. 2, Hummerland, and Gutman [54] for discussions of calcium.) When there is marked hypoalbuminemia, as in nephrosis and malnutrition, hypocalcemia also occurs. Usually, however, the ionized calcium does not fall, so that tetany rarely occurs in these situations.

The binding of thyroid hormone in the blood is not completely understood. About 85% of the protein-bound iodine of blood is found in the so-called "interalpha fraction" and only a small portion in the pre-albumin. A portion of the β -fraction has a specific ability to bind thyroxine and so may be compared to transferrin. When thyroxine labelled with radioactive iodine is added to serum, the radioactivity is found in the α -globulin. The capacity of this α -globulin to bind thyroxine is apparently limited in the same way as the capacity of transferrin to bind iron [55, 56], so that the conditions are similar for iodine and iron. The prealbumin also plays an important part [57].

Bilirubin in all forms is bound chiefly to albumin. Bennhold showed that certain drugs, such as atabrine, are also bound to albumin [37]. The same is true for penicillin, as shown by radioactivity studies.

French authors, notably Polonovsky and Jayle [58, 59] have made extensive studies of a protein fraction belonging to the α_2 -globulins. These authors found that on addition of small amounts of hemoglobin this latter substance formed a complex with an α_2 -globulin which they therefore named haptoglobin (= that which captures globin). This complex showed peroxidase activity and this fact was used for quantitative determinations. It was found by the French workers that haptoglobin (Hp) was much increased (normal values around 100 mg/100 ml) with maximal values of 1% or even more in many pathological conditions such as acute infections, necrosis (infarction) and malignancy. They demonstrated that the increase in α_2 -globulins seen in these conditions is chiefly accounted for by haptoglobin. The importance of haptoglobin determination may therefore be compared to that of the ESR.

Recent work in Sweden by Laurell and Nyman [60] has shown that the finding of a low haptoglobin value may also be important both for the clinical analysis of patients and theoretically. Nyman studied the disappearance of the Hb-Hp complex from the blood after the injection of hemoglobin (Hb) in an amount that corresponds to the normal hemoglobin-binding capacity of the serum. There was complete disappearance after ten hours. Renewed injection of the same amount of hemoglobin at this stage gave immediate hemoglobinuria, whereas the first injection caused no such event. Hemoglobin up to a certain threshold is obviously bound to haptoglobin in the blood and therefore no hemoglobinuria appears. The so-called renal threshold of hemoglobin is therefore in reality explained by the binding of two large molecules, the resultant compound being incapable of passing the glomerular membrane.

Smithies [61] performed starch gel electrophoresis of serum and found that normal sera could be separated into different groups according to their inherited Hp pattern.

The molecular weight of Hp is dependent on the group to which it belongs (Jayle, Boussier, Laurell); the molecular weight of the smallest Hp molecule being about 85,000. The reaction between this Hp (type 1-1) and Hb is often molecular. The molecular weight of the complex is thus about 155,000. The Hp 1-1 contains about 21% carbohydrates of which 45% is sialic acid.

Nyman has made extensive studies of the serum haptoglobin level in hemolytic conditions and has found that it is usually very low [62]. It has been found that haptoglobin changes are very sensitive and rapidly appearing indications of increased hemolysis. The present writer's rather extensive experience indicates that they are invaluable in the clinical evaluation of a hemolytic process. It seems to be of great interest that the *whole* Hb-Hp complex disappears from the circulation. This is different from, e. g., the iron bound to transferrin. The fate of the complex is not yet known. When the Hp is saturated, methemalbumin (Hamilton Fairley) appears. This substance gives the serum a brownish appearance with an absorption band in red that was for a long time regarded as hematin [63].

Lipoproteins

Of special importance is the transport of substances which are themselves not soluble in water but which must be kept in solution in the blood. This is especially true of the fats. To a certain degree these are carried in the blood as lipoproteins, but the largest amount of dietary fat is carried in the blood in the form of colloidal particles, chylomicrons. The lipoproteins are therefore not the dominant transport form of neutral fat. Cholesterol is in part transported as a lipoprotein. The manner of binding of lipid and protein is not yet clear.

The three methods of study of blood proteins in general have also been used for the study of lipoproteins: electrophoresis (Tiselius, paper, starch, etc.), ultracentrifugation (with sedimentation or flotation), and separation of protein fractions by Cohn's method.

The first investigator who was interested in protein-bound fats or lipoproteins was Macheboeuf [64], who in 1929 fractionated a protein in horse blood which had a constant fat content, "coenapse" (see vol. 2, Zöllner). This fraction was free of cholesterol and was found to be an α -globulin. Next, Blix and Tiselius [65] found that, in human plasma, both the α -fraction and the β -fraction of the globulins contain important amounts of fat. They also found small amounts of fat in the γ -fraction and in albumin. This conclusion was confirmed by later experiments.

Pedersen [66] investigated the size of the molecules of various blood proteins by means of the ultracentrifuge and found that one specific component, which he designated "X", was rich in fat. By electrophoresis, this fraction is largely a β -globulin and belongs to Cohn's fraction III-O. Pedersen made studies of the properties of this fraction and found that the slow sedimentation could not be explained by a low molecular weight. The molecular weight, in fact, was quite large: 10^6 . The specific gravity of the molecule, however, was virtually the same as that of the solution, 1.03. Gofman and coworkers in Berkeley, California, pursued the problem further and showed that it was easier to analyze the flotation of the light, fat-containing particles by means of ultracentrifugation if one dilutes the plasma with saline solution of suitable strength. (In this regard, certain terms should be explained. S_{20} refers to the sedimentation consts expressed ant at 20° and in Svedberg units, "S". S_r , on the

other hand, does not refer to sedimentation but to the opposite, flotation, and is used only in referring to lipoproteins, for these are the only proteins which rise to the surface.) By using various dilutions, the Berkeley group was able to show that there is a whole range of molecular sizes among these proteins with S_f values between 20 and 40. It still remains to be determined whether these data correspond with actual chemical differences or whether they are merely transient effects of varying aggregations of molecules. Gofman and his coworkers assumed that there is a correlation between certain flotation values and certain diseases. Thus, they compared the flotation pattern in patients with atherosclerosis with that in normals, and found that atherosclerosis, especially coronary, was associated with an especially high content of S_f 11-20 proteins. This fraction is therefore thought to be involved in the development of atherosclerosis of the blood vessels [67, 68]. However, the concept is not yet final: it is not yet known to what degree these observations are of real importance in understanding the pathogenesis of atherosclerosis.

Electrophoretic fractionation of the lipoproteins seemed to be of importance for clinical purposes, and several relevant investigations have been reported in recent years. The relationship between the α -lipoproteins and the β -lipoproteins was particularly investigated. It should be pointed out that these fractions do not comprise a single, chemically uniform substance, but that there are several α - and β -lipoproteins which can be separated by serologic methods [69].

A number of disorders in which the lipid content of the plasma is increased were studied by electrophoresis and by Cohn's fractionation method. It was found that the β -lipoprotein fraction was usually markedly increased, while the α -lipoproteins were often diminished. Several authors therefore concluded that a change in the ratio of β - to α -lipoprotein probably predisposes to the development of atherosclerosis. Such changes in the serum proteins were also found in nephrosis, myxedema, and certain lipidoses, diseases which are known to predispose to atherosclerosis. (Those lipoproteins which belong to the important group S_f 11-20 accompany the β -lipoprotein fraction.) Barr and his associates have shown that administration of estrogenic substances has a profound effect on the distribution of the plasma lipids among the various protein fractions. The cholesterol content of the β -fraction decreases and that of the α -fraction goes up. When the estrogen is stopped, the changes disappear [70]. Similar observations were made by Malmros et al. [71]. Swahn developed a method for the paper electrophoresis determination of the lipoproteins [72].

The observation that heparin clarifies lipemic serum has been thoroughly investigated [71]. Heparin influences the size of the aggregates of lipoproteins, and the clarification seems to be due to a reduction in the size of the particles [74]. The entire mechanism is not yet worked out, but other factors, such as heparin cofactor (which is necessary for the fluidity of blood) are also involved [75].

During the last year (1960) some important observations regarding an inherited disease with a complicated symptomatology (ataxic neuropathy, pigmentary degeneration of the retina, malformation of erythrocytes, acanthrocytosis, sometimes intestinal malabsorption) have been explained as caused by a deficient function of β -lipoprotein. The trait is apparently inherited recessively. A parallel with other "abiotrophies" of the central nervous system is near at hand and further research along similar lines seems most promising [76].

A further transport function of the lipoproteins depends on their ability to bind fat-soluble hormones and vitamins in the plasma. The transport of estrogenic hormones is apparently due to a specific β -lipoprotein. Vitamin A and vitamin E are also

carried in the β -lipoprotein fraction. The importance of these functions in endocrine pathologies is as yet quite unexplained, but this promises to be a fertile field for future studies. Recently a transport function has been postulated in the α_1 fraction.

Glycoproteins

Recently there has been much interest in carbohydrates which are bound to the serum proteins, especially as regards the importance of these "*glycoproteins*" in tissue inflammations and necrosis. In 1930, it was known that a so-called "acute phase globulin" was present in increased amounts in acute infections [77]. Hedlund investigated this fraction and found that he could determine its presence and amount by its property of agglutinating certain types of pneumococci. Later, it was shown that the "C-reactive protein" described in America was identical with or very closely related to this "acute phase globulin." The CRP has been crystallized and antisera prepared [78]. Serologic studies have shown that this protein fraction is probably not a normal component of plasma, but appears during infection. Hedlund made extensive studies on this subject [79] and showed that the acute phase globulin (APG) is increased in most febrile illnesses. It may also be seen in certain nonfebrile illnesses such as hypernephroma and chronic arthritis. The increase in APG occurs before the increase in the sedimentation rate. The increase of the α -globulin in nephrosis does not cause an increase of APG. The reaction is positive in acute myocardial infarction. Recent studies have shown that the APG is part of the γ -globulin fraction, rather than, as previously believed, the α -globulin fraction: these new studies include starch electrophoresis of serum and determination of APG in each protein band [80].

Heremans has recently shown that the so-called γ_x in immunoelectrophoresis is apparently identical with CRP. It is a rapid γ -globulin [81].

It was at first believed that a "carbohydrate-binding albumin" exists. Now, however, it is known that this glycoprotein is probably the result of inadequate separation of α -globulin from albumin during precipitation reactions. Thus, the fractions of McMeekin and Kekwick were shown by electrophoresis to be α -globulin rather than albumin. Other α -globulins with a high content of carbohydrate were studied by Blix and his group. They contain approximately 30% carbohydrate, chiefly as hexose and hexosamine. Blix in 1954 described sialic acid, which plays an important role in regard to these substances. Little carbohydrate is present in the γ -fraction, but carbohydrates are found in abundance in the α_2 -fraction and, to a lesser degree, in the β -fraction. Differences in the carbohydrate content are important for differential diagnosis in pathologic increases in serum globulin (hypergammaglobulinemia [56]). Thorough investigation of this subject by means of paper electrophoresis has been of great interest. The glycoproteins on paper can be oxidized by periodic acid, and the product of oxidation can be stained with Schiff's reagent. The amount of carbohydrate in pathological globulins is of great diagnostic importance [22].

Pedersen, in his ultracentrifuge studies of the components of serum, described a specific protein, fetuin, which contains a large amount of carbohydrate [66]. Electrophoretically this is an α -globulin. In the calf it comprises a large fraction of the serum proteins. Pedersen estimated its molecular weight to be 50,000 [66]. The important difference between the blood proteins of cow and calf points to a placental barrier: the several layers of the placental cell membranes confirm this fact. In man, on the other hand, where the barrier consists of only a single layer of cells, the composition of the blood of mother and child is approximately the same. Antibodies, as Vahlquist

has shown [83], can pass through the placenta. The large molecules of the α and β fractions, however, probably cannot pass through to the child, and this is also true for the isohemagglutinins. Later, however, antibodies do pass from mother to child in breast milk, and this occurs when synthesis of γ -globulin has not yet become active in the infant. Active production of γ -globulin probably begins in the fifth month of life. Conditions characterized by inadequate synthesis of γ -globulin are discussed on page 416. The important problem of γ -globulin formation in sterile (noninfected) animals is discussed in [84].

Amyloid

Among the disturbances of protein metabolism, the formation of *amyloid* is one of the most puzzling. Amyloid was first described 100 years ago and was extensively studied as a sequel of chronic infection, especially long-standing suppuration. Initially, it was believed that the deposits of amyloid in the liver, spleen, kidneys, and other internal organs were irreversible. Subsequently, it became possible to make exact clinical diagnoses of amyloidosis and to demonstrate amyloid in punch biopsies of liver and spleen; it was then found that amyloid might disappear when the underlying disorder responsible for its development improved. Thus, H. Waldenström [85] studied five cases of tuberculosis and showed, by means of such biopsies, that the amyloid subsided as the tuberculosis was arrested. Unfortunately, these studies have not been repeated with modern methods, so that nothing is known concerning changes in serum proteins during the development and subsidence of amyloidosis. At present it is rare to see severe fistulae in tuberculosis or other chronic infections, so that the study of this problem in amyloidosis is quite difficult. In cirrhosis of the liver and certain other diseases in which there is a reduction of serum albumin and increase in serum globulin, amyloid usually does not occur. On the other hand, amyloid is not infrequent in multiple myeloma and has also been seen in chronic rheumatoid arthritis. The pathogenesis of so-called primary amyloidosis, in which the serum proteins are normal, is also obscure. Nutritional factors are of importance (for example, experimental administration of casein facilitates deposition of amyloid) [86]. These questions are made even more complicated because of the development, in amyloidosis, of kidney damage with amyloid nephrosis; there follows loss of protein in the urine, and secondary changes may thus occur in the serum protein picture. Hereditary amyloidosis is a puzzling condition with dominant heredity [87]. Portuguese authors have studied hereditary amyloidosis of the nervous system.

Serum Albumin

Disorders of albumin metabolism consist almost entirely of a diminution of the content of albumin in the body and are probably related to defective synthesis of albumin. Pathologic increase in only albumin does not occur. When hypoalbuminemia is present, there is usually a decrease in the total blood protein, for albumin comprises the largest fraction of the serum proteins. There are many cases, however, in which reduction of albumin is associated with marked increase of globulin. In such cases, the total serum protein may even be elevated (Fig. 70). Such changes may be seen, e. g., in cirrhosis of the liver, collagen disorders, and myeloma. The total serum protein is low in severe malnutrition and in nephrosis. The older literature refers

Table 70. Secondary alterations of the blood proteins. (Concentration in grams¹⁰⁰; normal total protein is 7.2 grams¹⁰⁰.) (Pedersen and Waldenström 1949 [70])

Case	Diagnosis	Precipitation			Tiselius Electrophoresis				Ultracentrifuge		
		Tot	Alb	Glob	Alb	α	β	γ	4.5	7	19-20
	Normal (g/o)	6.4-7.0	3.0-3.6		50-58	6-12	13-21	17-22	7.5-8.5	11-24	0-5
	(g/o)	7.2	4.6-5.0	2.2-2.6	3.6-4.2	0.4-0.9	0.9-1.5	1.2-1.6	5.3-6.3	0.8-1.7	0-0.4
K 1-10	Malnutrition (p. 466)	2.8	1.1	1.7	0.7	1.1	0.5	0.5			
2-10		4.3	2.1	2.2	1.7	1.1	0.7	0.8	2.7	1.4	
3-20		6.6	4.1	2.5	3.4	1.3	1.3	0.6	5.1	1.2	0.25
3-25		7.3	4.8	2.5	3.8	1.0	1.0	1.5	5.8	1.0	0.5
N	Sprue, Jejunitis, Fatty liver	3.5			0.7	0.7	0.5	1.6			
A	Nephrosis, Lupus, eryth.	7.0	1.8	5.2	1.6	0.7	1.1	3.6	3.0	2.5	1.5
J	Kimmelstiel-Wilson	6.5	3.1	3.4	1.6	2.4	1.1	1.4	4.6	1.4	0.5
G	Cushing's Disease	7.1	4.9	2.2	4.2	0.5	1.3	1.1	5.5	1.5	0.1
D	Nephrosis	4.5	2.1	2.4	2.0	1.4	0.5	0.6	3.0	0.6	0.9
L	Xanthoma tuber, Angina pectoris	7.6	5.0	2.6	3.7	0.7	1.8	1.4			
S	Myxedema	8.5	4.0	4.5	3.4	0.7	3.4	1.0	5.6	2.9	
H	Biliary cirrhosis	8.6	5.2	3.4	2.4	0.9	3.4	1.7			
A	Hepatic cirrh.	10.8	2.8	8.0	2.6	0.4	0.8	7.0	3.7	7.1	—
V	Lymphogr. ven.	10.3	3.8	6.5	3.2	0.7	0.9	5.5	5.6	4.7	—
A	Kala azar	8.5	2.4	6.1					4.1	3.5	0.9
F	Lymphosarcoma and purpura	9.4	3.9	5.5	3.4	1.1	1.4	3.5	6.7	2.7	—
E	Sarcoidosis	12.0	4.1	7.9	3.5	0.8	1.8	5.9	5.4	6.6	—
H	Sarcoidosis and purpura	9.3			3.4	1.0	1.2	3.7	6.2	3.0	0.1
G	Chronic sialoadenitis	9.2	4.4	4.8	3.7	0.6	1.8	3.1	6.1	3.0	0.1
K	Lupus erythem. and sialoaden.	8.9			Paper						
					3.0	0.9	0.8	4.2			
G	Lupus erythem. diss. & purpura	10.3	4.0	6.3	3.3	1.0	1.1	4.9	6.8	3.5	—
J	Lupus erythem. diss.	8.7	4.4	4.3	3.6	1.0	1.0	3.1	6.3	2.4	—
P	Lupus erythem. diss.	9.1	3.3	5.8	3.7	0.7	0.9	3.8	6.7	2.4	—
P	Lupus eryth. diss.	10.6	3.6	7.0	3.2	1.4	4.2	1.8	5.0	5.0	0.6
J	Agammaglobulinemia	7.4			Paper				7.4	0	0
					5.2	1.0	1.2	0			

constantly to the albumin/globulin ratio (A/G ratio); this value of itself, however, is worthless, for there are many different disorders which may produce the same A/G ratio. It is always important to report protein values in absolute values, not merely as a ratio.

The most severe disturbance of synthesis of albumin leads to absence of this fraction. In 1954, Beunhold [88] reported two patients with complete absence of albumin in the blood. This condition is a hereditary abnormality as it occurred in a woman and her brother whose ancestors were related (two brothers had married two sisters and their offspring were the father and mother of the patients). The accompanying symptoms are now well known. Beunhold's first patient was a 30 year old woman whose blood was subjected to electrophoresis in order to explain an elevated sedimentation rate. This test disclosed that the serum was lacking in albumin. The globulin fractions were nearly normal. The patient also showed a positive Takata-Ara reaction, hypoalbuminemia of $8 \text{ mg}\%$, and a congo red disappearance of 61% . There was little edema, no signs of hepatic or renal disease, and normal somatic and mental development. When albumin was given intravenously, it disappeared from the circulation at a slower rate than normally. The globulins were somewhat increased and this must have sufficed to maintain the colloid osmotic pressure. The brother had the same symptoms. This finding is unique except for some very recent observations in Great Britain, U.S.A., and Switzerland. Another hereditary disturbance of serum albumin is the split albumin that was first described by Knodel in Germany [81] and has also been found by Gitlin in the U.S.A. No clinical symptoms are found. It has been reported in at least six kindreds. It seems probable that the inheritance is dominant and that there is an equal production of normal albumin and of the pathological albumin B. Homozygotes are not known.

Our knowledge of the reasons for and consequences of a reduction of serum albumin is quite extensive. Small decreases are very common. The study of malnutrition gives important data in this regard. Such studies originally suggested that there is a lower limit of serum albumin below which "hunger edema" occurs, but later studies failed to confirm the existence of such a critical level of albumin. There are cases of malnutrition with severe edema but without marked reduction of albumin, and, on the other hand, cases with severe hypoalbuminemia do not necessarily show much edema [88]. Various substances may be of therapeutic value; for example, vitamin B₁. Together with Pedersen, we have observed a case of severe hypoproteinemia following a period of inanition which was a sequel to typhoid fever [90]. The patient had been unable to eat for several months and was brought to the hospital severely ill. His total serum protein measured 2.8 grams/100 cc. and the distribution of the various fractions is given in Figure 74 and Table 70. Both albumin and γ -globulin, determined by Tiselius electrophoresis, were markedly reduced, but, on the other hand, the α and β -globulins were unchanged. All our efforts to increase protein synthesis by means of infusions of plasma, a diet rich in proteins, etc., were of no avail. The weight of the patient increased because of the development of severe edema. Diuresis could be induced with mercurial diuretics. One day, the patient placed his fiancée's picture upon his night-table; from this time on, his inability to take food subsided, and his appetite became excellent. At first, because of decrease of edema, there was a loss of weight. The serum proteins rapidly returned to normal (Fig. 74) so that, in six weeks time, they were approximately normal. Wallenius [91] investigated the production of anti-typhoid antibodies in this patient (Fig. 75). During the protein-poor, hypoproteinemic period, the titer of antibodies was low; when γ -globulin increased, the

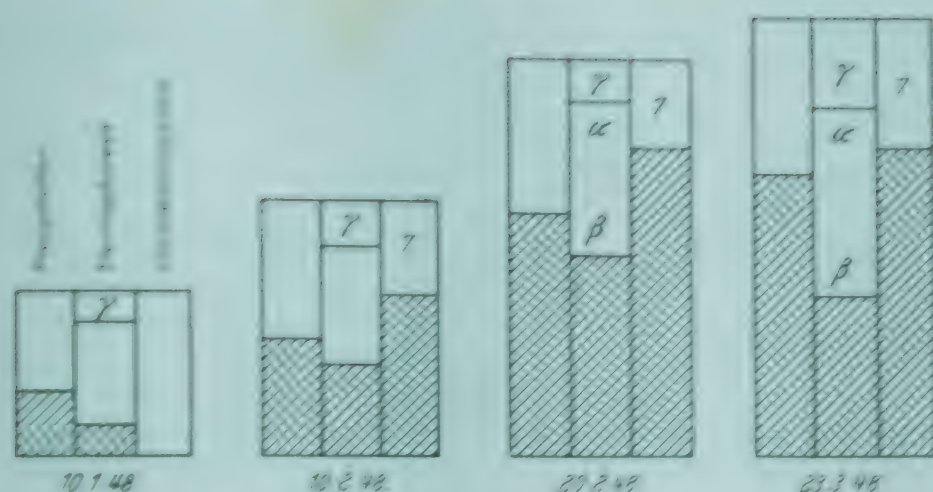


Fig. 74. Regeneration of the various serum proteins in a patient with nutritional edema. The lowest value for total proteins (left) is 2.8% the highest (right), 7.3%. (After Pedersen and Waldenström.)

also increased to abnormally high values. The observation made by Waldenström and Pedersen (20) confirmed by us in various other hypoproteinemias stated that there may be a reduction of albumin and γ -globulin without a major disturbance in the synthesis of α - and β -globulins, is probably of great importance.

Another disorder in which there is marked reduction of the total serum protein is nephrosis. Epstein was the first to suspect, in this condition, an interrelationship among the loss of protein in the urine, hypoproteinemias, and edema. The precise relationship, however, is not clear. Even in severe proteinuria, it is possible that the loss of protein alone is not responsible for the decrease of protein in the blood. A urinary albumin content of 1.5 to 3.0% in the presence of the usual oliguria of nephrosis means a protein loss of less than 15 grams per day. Neumenbruch (92) speaks of "nephrosis without nephrosis," by which he means the blood protein picture of nephrosis, but without albuminuria. The nephrotic syndrome must be explained partly as due to internal disturbances, especially in the synthesis of albumin. The mechanism of the peculiar spontaneous disappearance of edema occurring with the sudden onset of a marked diuresis is as yet unexplained. It is perhaps the end of a diuresis inhibition. The therapeutic effect of ACTH, which is often seen after discontinuation of the drug, as well as the diuresis in occasional cases after the use of nitrogen mustard, show that hormonal and other endogenous influences can cause sudden alterations in body hydration.

It is difficult to state whether cessation of ACTH or administration of cortisone causes diminished production of aldosterone in the adrenal cortex, and thus promotes diuresis.

There is clearly a definite parallel in most cases between the decrease of serum albumin and the development of edema in nephrosis. On the other hand, there are cases in which the serum albumin is low but no edema is present, notably in disseminated *lymphocryptocytosis*. In this disorder, one may see extremely low serum albumin levels, with marked increase in the serum globulin, so that the quantitative serum protein pattern resembles that of myeloma. However, a tendency towards the development of edema is not always present. There are other cases of doubtful diagnosis in which one sees marked hypoalbuminemia without much edema and without

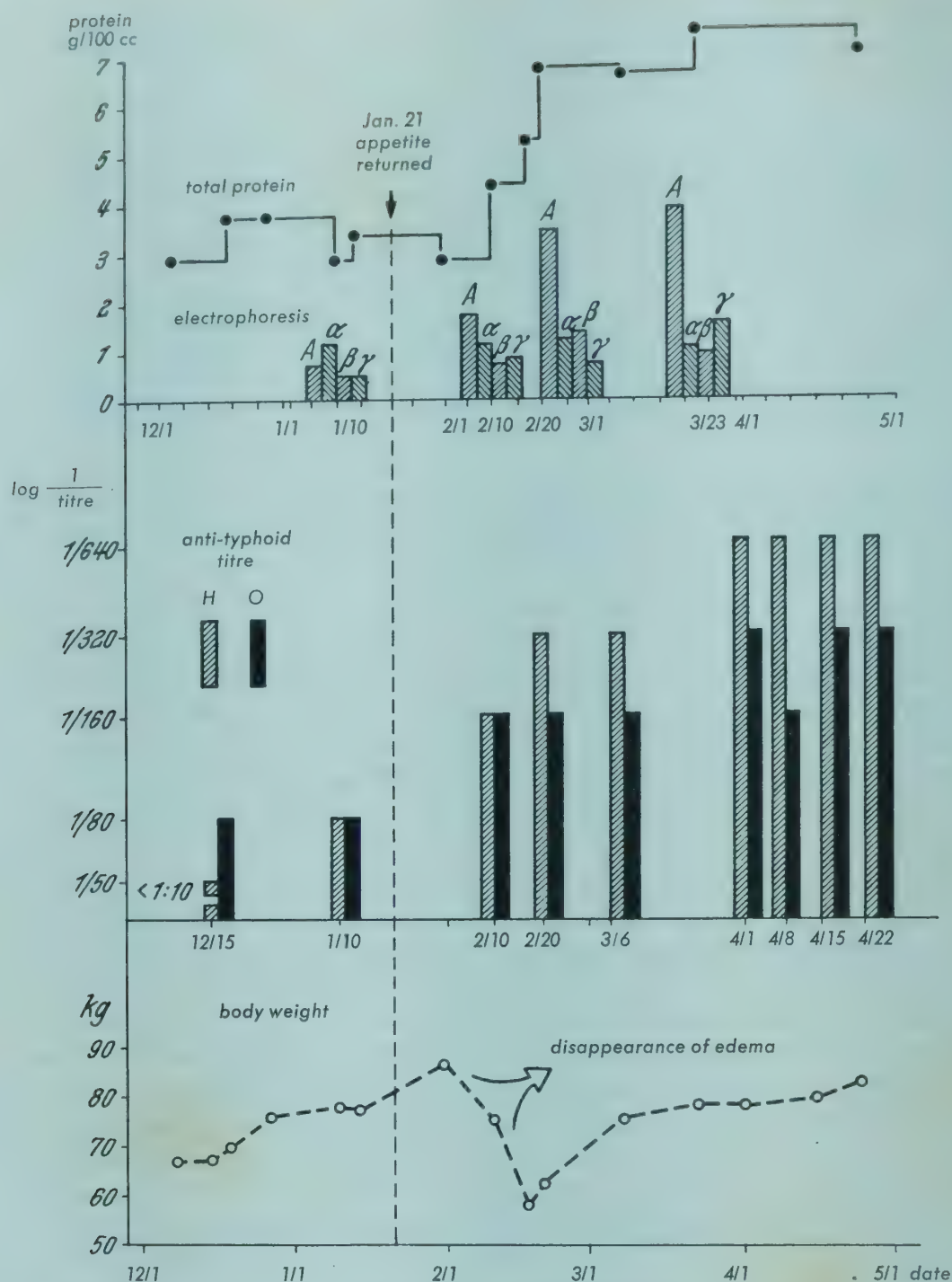


Fig. 75. The production of antibodies and certain other clinical observations in a patient with nutritional edema (same patient as in Fig. 74). (After Wallenius [63].)

proteinuria. In these cases, probably, the ability of the liver to synthesize proteins (albumin) is of basic importance. Studies which employ isotope-labeled proteins will be of value in explaining such cases.

The hypoalbuminemia of *myeloma* is discussed elsewhere.

Cirrhosis of the liver has long been an outstanding example of a disease with hypoalbuminemia. This feature is of importance because of its role in the development of ascites. As already discussed, a positive Takata-Ara reaction as well as other ab-

normal serum protein tests are caused by a reduction in serum albumin with concomitant rise in serum globulin. The reduction in albumin is thought to be related to defective formation in the damaged liver. It is difficult to state, however, if the reduced formation of albumin is a primary defect or if it is secondary to an increase in synthesis of globulin. There are, however, cases of cirrhosis of the liver in which the globulin is only slightly increased, although there is marked reduction in albumin. Such cases, of course, are in favor of a primary disturbance in the synthesis of albumin. In the natural course of hepatitis, there is first a reduction of serum albumin and α -globulin, as in many other acute diseases, and only later do the β - and γ -globulins rise [93]; these facts suggest that the decrease in albumin is not compensatory, but is a result of damaged synthesis of albumin. This problem too will lend itself nicely to solution by means of isotope-labeled protein fractions [94]. The excellent work by Freeman and others who studied the turnover of marked (I^{131}) albumin in a subject with analbuminemia should be consulted [95].

The relationship of the low serum albumin in hepatic cirrhosis to the development of edema is not entirely clear. Hypoalbuminemia is usually given as one of the three causes of the development of ascites, together with portal hypertension and antidiuretic factors. It must be recalled that often the administration of even large amounts of albumin has virtually no effect on ascites.

In certain types of chronic liver damage, e. g., *biliary cirrhosis*, there may be no reduction of serum albumin, and the only pathological finding in the serum proteins is a marked increase of the β -fraction, which must be related to the increased fat content of the plasma with formation of xanthomata [96]. In such cases, the serum is clear rather than milky — because of the high levels of lecithin. Other disturbances of hepatic function, e. g., *cardiac cirrhosis*, may lead to reduction of serum albumin with hypoproteinemia. This is especially the case in constrictive pericarditis with secondary hepatic damage. In such cases, Biörck et al. [97] have shown that successful surgery may be followed by return of the protein values to normal.

A reduction of serum albumin is found in a variety of infections and after trauma. The pathogenesis of this hypoalbuminemia is not clear, but it is tempting to explain it as a sign of liver damage. In such cases, the reduction in serum albumin is usually only moderate and disappears if and when the primary disease is healed.

Even in chronic diarrhea, the absorption of amino acids may be normal. In certain small-intestinal diseases, such as primary and secondary sprue, hypoproteinemia is often present, and this is also true in pancreatic diarrheas and in the rare intestinal lipodystrophy. In malabsorption, one must also consider the possibility of (gastrocolic) fistula formation with impaired absorption. We have encountered two patients with "jejunitis" with severe reduction of serum albumin, who also showed marked reduction of transferrin, ceruloplasmin, protein-bound iodine, and prothrombin, and also had hypocalcemia. The transport function can thus be markedly impaired in such cases. One may speak of a "strike of the stevedores." It is interesting to note that γ -globulin and β_2 -globulin, which is apparently related to γ -globulin, may also be quite low. Renal glucosuria is sometimes present. Recent American and Danish studies with isotopes seem to indicate that such conditions may be the result of protein losses through a damaged intestinal wall. This is probably the explanation of so called hyper-catabolic hypoproteinemia [98]. The diagnosis of such conditions is obviously quite important for the correct treatment of the patient (resection of "leaking" part of the gastrointestinal tract). The reader is referred to Gordon [99] and Gross [100] for recent reviews.

Unfortunately, *hypoalbuminemia* has often been overlooked when it occurs, as it sometimes does, in extensive weeping dermatitides. Severe hypoproteinemias may be found in pemphigus and in erythrodermia; it may be compared to that found in chronic burns. During the last war, there was great interest in the loss of protein following severe skin wounds, and replacement of protein was of great therapeutic importance. This is still a major surgical problem in, for example, burns.

Wuhrmann and Wunderly discuss "*essential hypoproteinemias*." In these cases, the hypoproteinemias are, of course, no more "essential" than is the "essential hyperglobulinemia" described by Waldenström. In many cases of hypoproteinemias, however, it is not possible to demonstrate any cause for the disturbance. Several cases of this type were reported by Wuhrmann and Wunderly; in one such instance, sarcoma of the small bowel was found at autopsy. The protein disturbance had been present constantly for some 6 years. The relationship between protein disturbances and gastrointestinal sarcomas is certainly very complex and is deserving of further study. It is possible that "leakage" plays a part in some such patients.

From the clinical point of view, hypoalbuminemia means a reduction of the colloid osmotic pressure of the plasma; even a marked increase in plasma globulin cannot compensate for the reduction in albumin (cf., however, [88]). Serum albumin is also of importance in wound healing; the impairment of albumin synthesis following severe trauma can disturb healing of wounds.

The Hyperglobulinemias

An increase of serum γ -globulin occurs in a variety of diseases, many with indefinite etiology. Pedersen and Waldenström have found marked hyperglobulinemia in the following conditions (1949 or earlier): xanthoma tuberosum, myxedema, biliary cirrhosis (all with lipemia and hyperbetaglobulinemia), lymphogranuloma venereum, kala azar, liver cirrhosis, sarcoidosis, sialoadenitis with and without chronic purpura, lupus erythematosus disseminatus. In all of these, the fractions showed broad bands (see fig. 76) whereas patients with myeloma, macroglobulinemia and essential hypergammaglobulinemia showed narrow bands (Fig. 76). Some of the so-called essential cases have died and post mortem did not reveal any signs of myeloma or other similar disease. These are therefore truly "essential."

As a model for the study of the changes in γ -globulin in a disorder of *known* etiology, Pedersen, Sonck, and Waldenström [101] have chosen cases in which a *virus infection* was the cause of severe hypergammaglobulinemia. It was well known that many cases of lymphogranuloma venereum show a rapid erythrocyte sedimentation rate and marked increase in serum globulin. The presence of virus in the body seems to result in increased formation of γ -globulin, and treatment is followed by reduction of the ESR and of the increase in γ -globulin. We thus see a longstanding immune reaction against the virus of lymphogranuloma, which can be interrupted by specific therapy. The hyperreactive increase of gamma globulin in so-called essential hyperglobulinemia (and in collagen diseases), on the other hand, is rarely reversible for any period of time. Similarly, in patients with cirrhosis of the liver, established hypergammaglobulinemia seldom disappears, and it is not known if this might be a reaction to the hepatitis virus, which may still be present in the body. The gamma fraction may also be increased in alcoholic cirrhosis of the liver, although usually less so than in posthepatic cirrhosis. The causes of these changes are not yet known, and most theories are quite vague. At present, however, the concept that autoimmunization may play a rôle in

these diseases has become quite popular. Marking has published interesting observations on so-called lupoid (after L.E.D.?) hepatitis [102]. We have also seen such patients and the author described in 1950 a number of young girls with liver carcinoma and an unusually high γ -globulin. In some cases steroid therapy has been of great help. The γ -band in cirrhosis, like that in lymphogranuloma, is broad. Kala-azar is another chronic infection in which there is increase in γ -globulin and reduction in serum albumin. In subacute bacterial endocarditis and in certain other chronic infections, the γ -fraction may also be increased (Fig. 19). All these conditions cause a broad-banded γ -globulin consisting of 7 S components.

Albumin α_1 α_2 β_1 β_2 γ

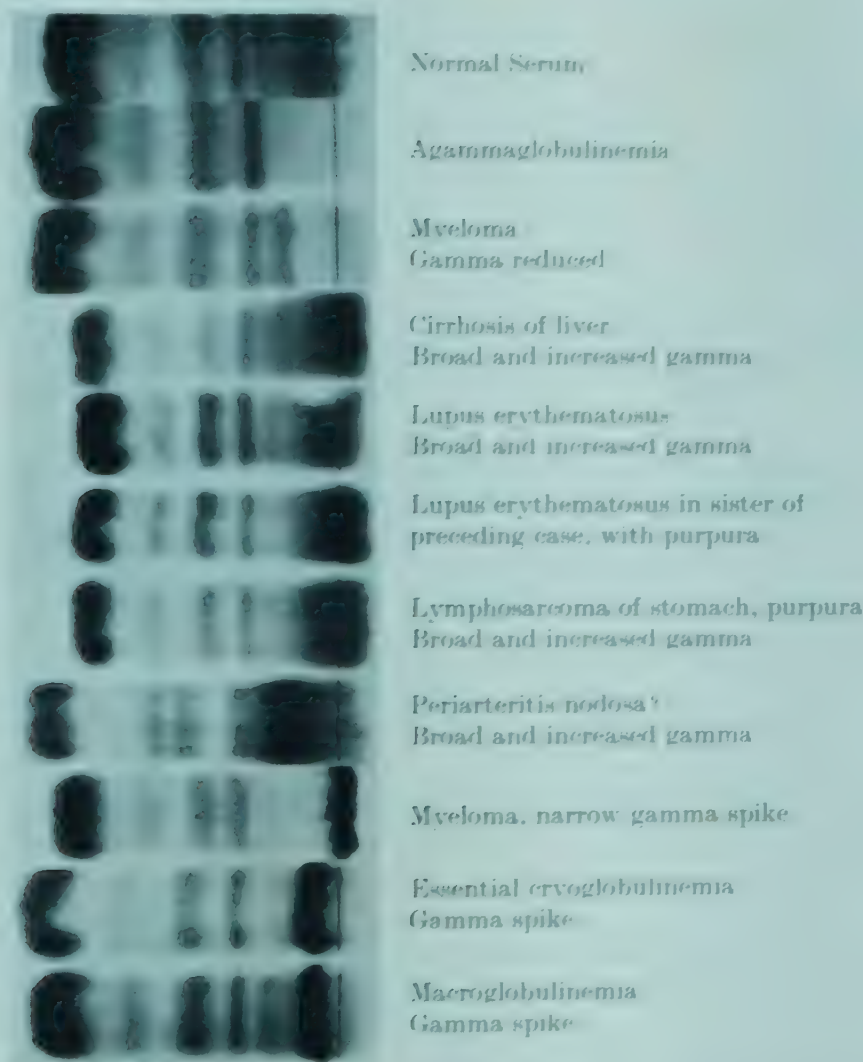


Fig. 76. Paper electrophoretic patterns of the serum of several disorders with disturbances in the serum protein pattern. Note the narrow bands in the last three sera in contrast to the broad bands in the five above these.

In acute infections, on the other hand, one usually finds an increase in α -globulins, and the same is true in tissue breakdown, infarcts, tumors, and fractures. Hypoalbuminemia is also common in such conditions.

Since about 1940, the author has followed a group of patients who showed peculiar disturbances in the pattern of serum proteins. Although these patients could not be

Table 71. "Essential" changes of the serum proteins. (Concentration in grams/100 ml of serum. (From Pedersen and Waldenström 1949 [70])

Case	Diagnosis	Precipitation			Tiselius Electrophoresis					Ultracentrifuge				
		Tot	Alb	Glob	Alb	α	β	γ	4.5	7	11	19	20	
N.	"Essential" hyperglobulinemia	8.0	5.1	2.9	3.3	0.6	2.8	1.3	6.0	2.0	—			
L.	"Essential" hyperglobulinemia and purpura	10.0	4.5	5.5	4.0	0.5	1.5 0.8	4.0	6.4	3.4	0.2			
T.	Myeloma (β lipo?)	11.4	3.2	8.2	2.1	0.9	6.7 0.2	0.7	4.9	6.5				
U.	Myeloma (β)	11.4	3.6	7.8	3.0	0.3	6.9	1.2	4.9	3.9	2.6	—		
O. E.	Myeloma (γ)	13.5	2.0	11.5	1.7	0.8	1.7	9.3	3.4	10.1	—			
J.	Myeloma (normal)	8.7	6.0	2.7	5.5	0.9	1.4	0.9	7.4	1.1	0.2			
L.	Macroglobulinemia I	11.5	4.4	7.1	3.3	0.6	0.8	6.3	5.3	0.7	5.5			
B.	Macroglobulinemia II	10.6	4.6	6.0	3.5	0.5	1.4	5.2	4.9	2.4	3.3			

diagnosed as having any of the diseases known to cause changes in the serum proteins, such as myeloma, cirrhosis of the liver, or arthritis, they have persistently shown an elevation of the sedimentation rate for over 10 years. There was usually an increase in the γ -globulin and a resulting high ESR; these were the prominent findings, and the disorder was therefore called "essential hyperglobulinemia" (Fig. 77). The course of these patients was carefully followed in some cases for over 20 years, and no clinical diagnosis has as yet been made [11]. It may be that this disorder represents a primary disturbance of protein synthesis *sui generis*, that is, a "molecular disease" of the serum proteins. Most of the patients, however, gradually develop various symptoms which could be interpreted as related to collagen disease. Today, we are inclined to consider many cases of "idiopathic hypergammaglobulinemia" with a broad γ -globulin band as instances of hyperimmune reactions. Thus, we have seen such cases in which the first signs consisted only of a slight normochromic anemia and leukopenia, with a high sedimentation rate. In the ensuing years, however, these patients developed one or more groups of symptoms which fit best into the picture of disseminated lupus erythematosus.

The most important observation seems to be the fact that hypergammaglobulinemia is the connecting link between all these cases, who later may develop disseminated lupus. This may be of fundamental importance for the understanding of such conditions (auto-antibodies in autoimmune disease).

In these patients with marked broad-banded increases in γ -globulin a number of different serologic tests were performed [103]. It was found that many of the sera showed agglutination of sensitized sheep cells in high dilutions, even when they lacked symptoms of arthritis. This positive reaction is in favor of a collagen disease, but is not diagnostic of rheumatoid arthritis. It is never positive, on the other hand, in myeloma or allied conditions. In this disorder, the only serological disturbance is a

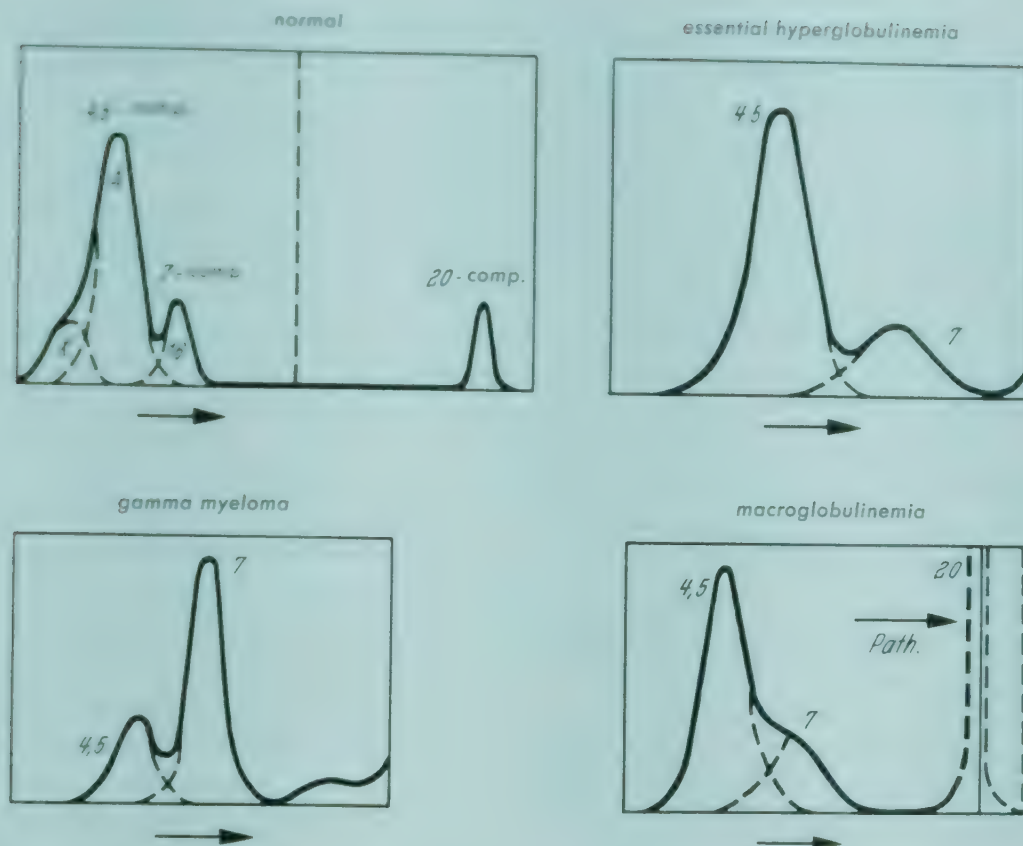


Fig. 77. Ultracentrifugation patterns. The arrow gives the direction of sedimentation. Normally, the 20 S-components do not exceed 5%. In gamma myeloma, there is an elevation of the 7 S-components. The pattern of essential hyperglobulinemia is that of case N. of Table 71. The macroglobulinemic diagramm was made after a period of centrifugation. (From Pedersen and Waldenström.)

nonspecific increase in the anticomplementary titre. Liver "cirrhosis" very commonly is accompanied by an increase in antistreptolysin titre and also shows high titres of rheumatic factor. Purpura hyperglobulinemica, chronic sialoadenitis and disseminated lupus are the three conditions in which we see a real galaxy of different positive serological reactions. False positive Wassermann reactions, however, were surprisingly rare in our experience [103].

One patient had hyperglobulinemia and chronic purpura, without other symptoms, for more than 10 years. She was considered to have "hyperglobulinemic purpura" [104]. Subsequently she developed an episode of high fever, renal insufficiency, and L. E. cells in the blood. This acute phase was treated with large doses of cortisone, and the patient then passed once more into the original chronic stage of hyperglobulinemic purpura with increased γ -globulin in the blood. The patient's sister has also shown a high ESR for a long period of time, but with no purpura. This sister developed a transient typical butterfly eruption (L. E.) on the face. Another sister had died of typical disseminated lupus. Still another (4th) sister has had a high sedimentation rate for a number of years, and shows a tremendous increase (3.4 gm) of the γ -globulin. She has always been quite well [105]. It is possible that the familial incidence of these illnesses is not as rare as previously believed, since we have seen several familial cases of this type. For a long period of time another of our patients was considered a

typical example of "essential hyperglobulinemia," until she developed an acute episode of renal insufficiency accompanied by a butterfly-rash on the face, and a diagnosis of disseminated lupus could be made. The acute episode passed off in several weeks, and for the past 4 years the patient has again been asymptomatic, showing only slight anemia and marked hypergammaglobulinemia.

In several of the patients with chronic benign hypergammaglobulinemia, there was also a chronic, orthostatically induced purpura (Waldenström [104]). Normochromic anemia and leukopenia were also common. Many workers feel that this reduction in the red and white cell counts may be due to a serologic mechanism - i. e., a hemolysin or agglutinin. It is just as likely, but very difficult to prove, that the purpura is the result of damage to the endothelial lining of the small blood vessels by antibodies against endothelial cells (Stefanini). In this way, it would be possible to explain the findings on the basis of autoimmunization. Thus, it is clear that in these cases the increase of the total γ -globulin is the result of increases in an entire series of different γ -globulins. This concept is illustrated by the width of the gamma strip on the electrophoretic pattern. In contrast, myeloma, for example, shows a narrow, high spike. We follow Riva, who calls this "M-type globulins" (M for myeloma or macroglobulinemia). Thus, some of these conditions with their pronounced broad increase in γ -globulins could be thought of as a reaction against certain, albeit as yet unknown, stimuli or could possibly be on a "hereditary" basis. Cf. the old clinical concept: constitution, diathesis.

Undoubtedly, one of the chief functions of those tissue cells which synthesize the gamma globulins is their ability to produce *antibodies* against given antigens. As already discussed, the body reacts to acute stresses by producing increased amounts of fibrinogen and α -globulin. Apparently, this represents a non-specific "defense" reaction. The true specific antibodies are found chiefly in the γ -fraction; some, such as the O-agglutinins against typhoid and the isoagglutinins of the blood, are present in the β_2 -fraction. However, many studies suggest that the so-called β_2 -fraction (which certain authors call the γ_1 -fraction) is biologically part of the γ -globulin rather than of the β -globulin. If one judges from the amino acid content of the two fractions, they show similarities which separate them into a group distinct from the other serum proteins [106]. In the ultracentrifuge, these fractions usually have the same sedimentation constant; and, in addition, their reactions to the cadmium test and to gelation (formol gel and alkazide) are also similar. They also seem to be related serologically. Their connection with diseases of reticulum cells and dysproteinemia is obvious.

Together with Kulneff and Pedersen, the author [23] has studied 3 sera in cases of hypo- and agammaglobulinemia by means of ultracentrifugation. These studies showed that all the γ -components were absent, as well as the gamma fraction (Fig. 78). The β -fraction could not be completely investigated, but it is reported that isoagglutinins are missing in agammaglobulinemia, although they belong to the β_2 - and not to the γ -fraction. It is therefore obvious that there is no clearcut demarcation between β_2 and γ , but that one should rather speak of γ_1 (= β_2) and γ_2 . In a case which was investigated later, the β_2 -fraction was found to be present, although in reduced amount. It seems definitely proved that β_{2A} (= γ_{1A}) is absent whereas γ_{1M} may vary from one case to another. (See Grabar and Burtin, 1960.)

Most important alterations in γ -globulin are seen in the so-called rheumatic disorders. Today, such disorders are considered by many authors to be basic diseases of the ground substance of the mesenchyme. The word "collagen disorder" thus implies

a primary disturbance of collagen. We do not really know if this concept is correct, and it would probably be more accurate to speak of a disturbance in the metabolism of the interstitial substance (e. g., of a "desmosis"). The word "collagen disease" is however well established, and I shall therefore use it until a better word is found.

Klinge's description of the rheumatic disorders was published in 1933 and has become a classic in the field [107]. He stated that the changes in the connective tissue of the body in the patient with a rheumatic disorder are much more widespread than previously thought. The most important point he made, however, was the proof that similar histologic changes are found in immune reactions. In 1941, Klemperer [108] showed that lupus erythematosus and scleroderma also had similar histologic pictures. In 1947, Rich [109], on the basis of the similarity of lesions produced by means of rabbit experiments utilizing protein antigens, propounded a new theory which united all these disorders. He also collected a series of clinical observations on the actions of certain drugs which had produced immune reactions in patients. In his opinion, the collagen disorders are instance of hyperimmunization; this concept would explain the changes in the gamma fraction very well.

In recent times, the chemical basis for the *Wasserman reaction* has been widely studied. The exact conditions are very complicated, but the newer technics with electrophoresis in firm substrates have permitted better separation of the reagins [110]. In normal serum, proteins were isolated in the γ -fraction and sometimes also in the β_2 -fraction which produced a positive Wasserman reaction. In the true syphilitic Wassermann reaction, the conditions are not always the same. In primary syphilis, reagins are found in the γ -fraction which are probably also concerned in the false positive Wassermann tests found in certain collagen diseases. Such biologically false Wassermann reactions were found for example early in disseminated lupus [111]. In the serum of patients with secondary or late syphilis, reagins were found both in the γ -fraction and among the β_2 -globulins.

It is not yet possible to determine all the factors responsible for the *coagulation of blood* by means of chemical methods. A number of different factors have been found to

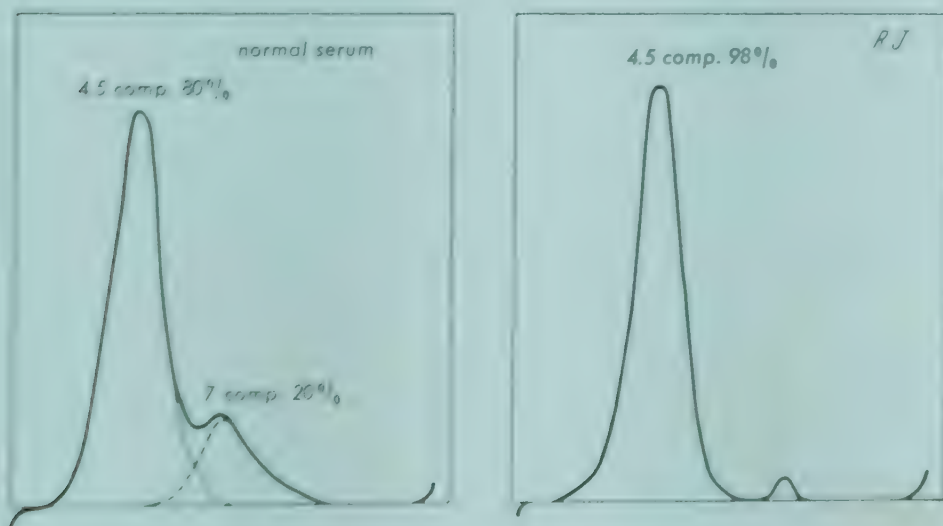


Fig. 7c. Ultracentrifugation diagrams of normal serum and of serum of a patient with agammaglobulinemia showing absence of the γ S-components. [After Kulneff, Pedersen and Waldenström.]

be involved in blood coagulation, but it is by their actions, and rarely by their chemical properties, that they have been identified.

Among the serologic abnormalities found in chronic collagen disorders are also disturbances in coagulation. The so-called "Hemmkörperhämophilie" was first defined by Deutsch [112] in dysproteinemia, and was interpreted as due to immunization against thromboplastin as a result of repeated transfusions. It was later shown that such disturbances of coagulation occur spontaneously in collagen disorders [113], and similar coagulation disturbances are seen following pregnancy. It is probable that the development of such specific circulating anticoagulants is another sign of generalized "increase" in the synthesis of γ -globulin, i. e., of antibodies. In several instances false positive Wassermann tests occurred together with antithromboplastin [114, 115, 116].

The whole problem of *fibrinolysis* is quite complicated and cannot be discussed here. A review is found in Sherry et al. [117]. From recent studies it is evident, how-

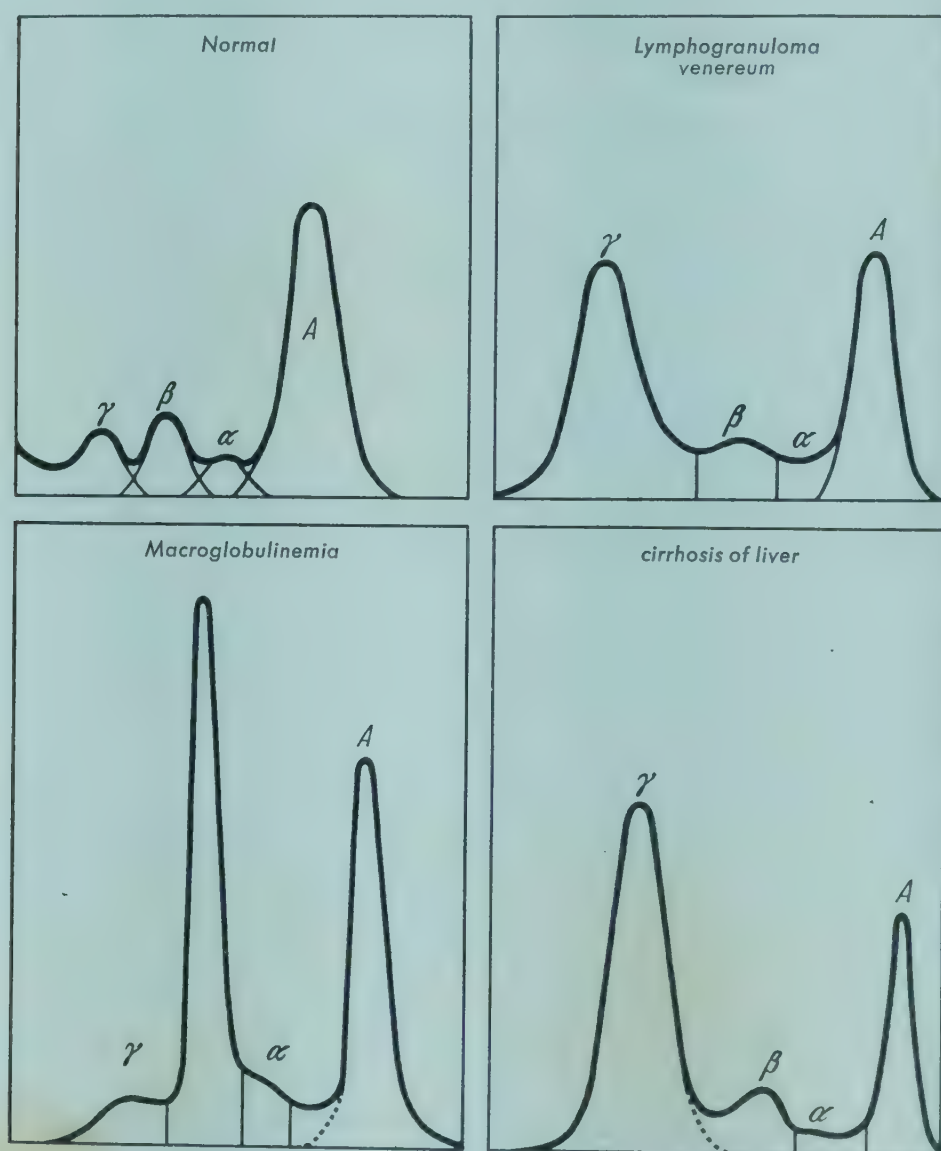


Fig. 79. Electrophoretic patterns showing in different disorders the increase of the short gammaglobulins.

ever, that fibrinolysis plays an important part in clinical medicine. Prostatic tissue as well as semen have fibrinolytic activity and this fact probably explains the fibrinolysis in metastasizing prostatic carcinoma and perhaps the bleeding after prostatectomy. Recent studies indicate that ϵ -aminoisocaproic acid is an inhibitor of fibrinolysis and can be used clinically to great advantage [118].

It seems probable that certain patients with an increased tendency to thrombosis have a defect in the inhibitor of the activator of plasmin function [119]. Impaired retraction of the clot is also seen in disturbances of the plasma proteins, for example in myeloma, but objective evaluation of this finding is difficult. A special type of hemorrhagic diathesis with mucous membrane manifestations is seen in macroglobulinemia. The two types of purpura, hyperglobulinemic purpura [104] and cryoglobulinemic purpura are discussed on pages 433 and 442. They are not disorders of blood coagulation, but are due to damage to the capillary endothelium. Congenital disturbances of body proteins which have to do with certain specific coagulation factors, have been discussed at the beginning of this chapter. Thus, there are several different coagulation defects which may be found when there is a disturbance of the blood proteins.

Complement, which has played such an important role in all serological discussions, is still difficult to grasp from a chemical point of view, and many authors considered complement simply an expression of some special function. In the last 10 years, following the demonstration that there are several substances involved in its function, complement has been identified chemically. Today, it is generally believed that there are 4 components to complement, 3 of which have been isolated in more or less pure form [120]. These are C_1 , a globulin, formerly called "middle piece"; C_2 , formerly called "end piece"; and C_4 . C_2 and C_4 are rich in carbohydrate and are designated as mucoproteins. C_1 comprises about 0.7% of the total serum protein of the guinea pig. The electrophoretic behavior of these component portions is not yet definitely established. C_3 has been related by Muller-Eberhard to the new protein fraction β_{11B} .

The complement titer of a given serum is defined according to that component which has the lowest titer. This is therefore the "limiting factor" of the function of complement. Normally, this statement applies to C_3 . The different components however do not have identical properties (for example, as regards passage through the blood-brain barrier); hence there are many diagnostic difficulties. The problem of properdin [121] is still being investigated and a definite opinion on this question is not yet possible. The reader is referred to recent reviews.

The anticomplementary activity of various pathological protein fractions has been thoroughly worked out. It seems well established that only certain members of the γ globulin fraction are anticomplementary; normal purified γ -globulin has some anticomplementary action. Pathological sera sometimes have a high titer of an anticomplementary thermostable factor.

Such a factor is found, for example, in the serum of some myeloma patients. However, other diseases with hypergammaglobulinemia may also show such anticomplementary factors in the serum—namely, the collagen disorders. Olhagen [122] has made detailed studies of the subject. The phenomenon has also been reported in kala-azar [123], lymphogranuloma venereum, and cirrhosis of the liver. The anticomplementary effect does not in itself depend on the amount of the γ -fraction: a serum with high γ -globulin may have no anticomplementary effect, while another with less γ -globulin may be strongly anticomplementary [103].

Myeloma

Myeloma is the outstanding example of a disease in which there is profound alteration of serum protein metabolism. The nature and position of myeloma, pathogenetically, is not yet fully understood. Pathologists believe that its destructive nature classifies it as a tumor with metastases. On the other hand, there are instances of myeloma with diffuse involvement of the bone marrow where X-rays show only osteoporosis. Myelomatous involvement of the bone marrow is generally widespread, and there is no primary tumor with metastases in most instances; both these facts are in favor of a "systemic" disorder of plasma cells. The combination of myeloma and plasma cell leukemia demonstrates the relationship of myeloma to the leukemias. Myeloma is thus best thought of as an autonomous unlimited growth of the plasma cells. The recent ideas of Burnet regarding myeloma as a somatic mutation of one definite clone of plasma cells are most interesting.

The role of plasma cells in the synthesis of serum globulin has been emphasized by various authors [124, 125, 126], on the basis of the increase in serum globulin during plasma cell proliferation. Experimental verification is difficult, but it is generally accepted that plasma cells and cells of the lymphatic series participate in the synthesis of serum globulins.

The disturbances of serum proteins in myeloma are manifold. Synthesis of serum albumin is never increased – a strong argument that plasma cells are not involved in albumin synthesis.

There has been considerable discussion regarding the subdivision of myeloma according to dominant electrophoretic fractions. Many modern writers speak about α , β and γ myeloma without being able to make a clinical distinction between these different groups. It is evident that the migration velocity of the pathological globulin in the electric field might not necessarily be correlated with important biological differences. Recent developments in the field of immunoelectrophoresis by Grabar and his school in Paris, by Swiss authors, and by Heremans [81] seem to prove that what the present author has previously called the γ family should be redefined as the γ system (Heremans), comprising three different groups on immunoelectrophoresis. Heremans has shown that one of these groups which was previously called β_{2A} by the French authors really belongs to the γ system, even if some of its members may have a velocity that is so rapid that the globulin is characterized as α_2 ! In a joint program with C.-B. Laurell and the present author, Heremans has shown that several pathological α fractions in myeloma cases really belong to this immunoelectrophoretic " β_{2A} " fraction [127]. According to the present author, it seems much more appropriate to adhere to the terminology first propounded by Kunkel and his school [138] and call this fraction γ_{1A} .

The proteins in multiple myeloma would then all belong to Heremans' γ system; i. e., they would be biologically closely related even if some of them have a very rapid mobility. This obviously makes sense insofar as plasma cells are always proliferating in myeloma and are also normally connected with the synthesis of antibodies belonging to the biological γ system. According to this opinion myeloma cases could be divided into only two main groups: γ myeloma, always with typical γ mobility and immunoelectrophoretically belonging to the γ line; and γ_{1A} (" β_{2A} ") myeloma, where the mobility of the pathological fraction may stretch from rapid γ as far as slow α . It is probable that these new protein individuals must be rebaptized as our knowledge increases. The third group in the γ system is what Grabar has called the β_{2M}

fraction, where M stands for macroglobulin. This fraction also occurs normally and corresponds to the γ macroglobulin described by Pedersen [66] and Waldenström in macroglobulinemia [145]. The name of this fraction should be changed to γ_{1M} and this would mean that a biological γ system comprises three immunoelectrophoretic groups: *sensu strictiori* $\rightarrow \gamma_{1A} + \gamma_{1M}$. As will be discussed later, patients with marked increase in γ_{1M} have a special clinical picture called macroglobulinemia. At present I shall only treat the clinically typical myelomas.

There is no longer any discussion about the fact that there exist myeloma patients with marked isolated increase in α_2 -globulin and a typical clinical picture. These cases, however, are very rare [128, 129]. There are also patients in whom the pathological component migrates as a β_1 or a β_2 -globulin, and in addition there is the very large group of patients with typical γ mobility (slow or rapid) of their pathological globulin. In spite of the great variability in the mobility of the pathological fraction there are always two constant features. The pathological fraction *never changes its mobility*, even if it increases very much in quantity; and, another important feature, *it never disappears* [11].

Burnet has proposed the interesting hypothesis that myeloma could be explained as a proliferation of only one clone of plasma cells through a somatic mutation. This clone would produce one, or perhaps a few, pathological globulins which would always remain the same. The author has discussed this clonal theory in a recent paper [130]. It is evident that the change in protein synthesis is irreversible and remains qualitatively constant. There are, however, certain important features which must be explained. A number of patients with myeloma do not have any discrete increases in a special γ fraction. Their protein pattern is characterized by a low γ globulin. Some of these patients have a marked protein loss through the urine; others lack proteinuria but are still markedly hypogammaglobulinemic. This condition is of course difficult to explain. It is obvious that the normal γ fraction is often defective in myeloma, with a resultant tendency to infections. This is also true in many instances of benign essential hypergammaglobulinemia of M type and in lymphatic leukemia.

Another question that complicates matters is the occurrence of patients with marked increase in one special γ fraction without any signs of myeloma. Such patients have been followed in Sweden for many years without the development of myeloma. Some of them have died from some intercurrent disease after the condition has lasted for many years, and at post mortem there were no signs of myeloma. This must mean that a picture electrophoretically indistinguishable from what we see in myeloma may be found as a completely benign condition in elderly people. It is interesting that the age incidence is about the same as in myeloma and that a certain liability to infections is probably present. It may be expected that this is caused by deficient formation of "normal" antibodies belonging to the γ family. Important differences seem to be that there is no progression of the hypergammaglobulinemia during the years, nor is there hypoalbuminemia. Anemia may be present, however. It is difficult to tell if some of these patients may or may not have "premyeloma" [131].

The reader may well ask if there are any clinical or cytological differences between patients with a typical γ -globulin myeloma and a γ_{1A} myeloma. We have tried to establish such differences in our myeloma material from Sweden but only a few points are worth mentioning. There has long been much discussion regarding the cytology of myeloma. Wallgren was the first to point out that we should speak about one myeloma cell and not try to divide these into a number of different types as had been done before. This was a great simplification and later authors have always talked

about myeloma cells or plasma cells without much further subdivision. Gormsen has shown that 4% is the upper normal value [126]. A number of attempts at cytological classifications of the cells have been described recently but without much success. We believe, however, that the cells in γ_{1A} myeloma may have some specific characteristics. In a paper with Heremans and Paraskevas the author has discussed some cytological properties that so far seem to exist only in γ_{1A} myelomas [132]. Bone marrow smears from 12 such patients were examined. It was found that two of these patients had no special characteristics. In the other ten one or more of the following three phenomena were always present: (1) numerous plasma cells with a "flaming" (i. e., eosinophilic) cytoplasm; (2) numerous cells with a small pyknotic nucleus and a very large cytoplasm which was often divided into compartments that gave an impression of storage. We therefore called these cells thesaurocytes; and (3) intranuclear inclusion bodies which contain Schiff positive material. Such inclusion bodies had already been described in macroglobulinemia by German and American authors [133, 134]. They are also found in the nuclei of γ_{1A} myeloma cells. This is of interest since γ_{1A} globulin has a high content of carbohydrates like macroglobulins. Further research will be necessary before these questions are definitely clarified.

Our knowledge regarding any functional properties of these pathological fractions is very scanty. It is known, however, that proteins usually regarded as antibodies may be found in the γ , γ_{1A} and γ_{1M} fractions. The function of one of the β globulin fractions is to carry lipids. Curiously enough a number of myeloma patients with very marked lipemia have been reported [11, 81, 135]. Their pathological globulin fraction always had the migration velocity of β -globulin and in two instances Heremans succeeded in demonstrating that this globulin was an immunoelectrophoretic γ_{1A} globulin. Many facts however are against the obvious assumption that the myeloma cells in these patients simply produce normal β lipoproteins in increased quantities. This is of course a very important and interesting group of myelomas which deserve further investigation.

One problem which is most intriguing is the following. If we accept the theory that there is a clonal selection in antibody production, it seems probable that each abnormally increased protein in myeloma should correspond to an antibody. In view of the enormous number of clones, therefore, a large number of antibodies must also be assumed. It is probable that the specific antibody has a fair chance of never being discovered with the limited number of testing methods that we now have.

In addition to electrophoresis, it is possible to use the ultracentrifuge to define the pathological serum proteins in myeloma. Fig. 77 shows the results of such studies in the first series of sera examined by Pedersen and Waldenström before 1950 [90]: of those having pathologic γ -components, almost all showed an increase of the 7-S component. Certain cases were more complicated. Thus, in one serum, the tremendous increase of γ -globulin was associated chiefly with an increase of a globulin whose sedimentation constant was 11-S. Two myelomas with increases of β -globulin also showed an 11-S component. In no cases of myeloma did the serum show pathological increase of the 20-S components, and in many cases these components were entirely absent. In rare instances deviations from normal could be found neither by electrophoresis nor by ultracentrifugation. A decrease of normal γ -globulins is a common finding. Several authors believe that this situation indicates a loss of protein in the urine due to more marked Bence-Jones proteinuria, but a normal blood protein picture may also be present in the absence of proteinuria. Neither histologic nor clinical differences could be found in these various types of protein disturbances. The rare

cases with a typical clinical and cytological picture and without any disturbances in the serum protein pattern are especially difficult to explain.

It is difficult to evaluate the true nature of the pathologically increased protein fractions found in myeloma. Certainly, in the future, serologic methods will be of value, but the present problem lies in the difficulty of obtaining pure protein fractions for antigen studies. Kunkel, Slater, and Good [136] have prepared a reliable anti-gamma serum. Wuhrmann, Wunderly and Hässig [137] performed experiments in 1950 in this field, and found immunologic specificity only of the increased β -fractions, but not in γ -myelomas. In 1955, Slater, Ward and Kunkel [138] published a paper on the serologic differentiation of the proteins in myeloma. Investigations utilizing antisera prepared against 10 isolated protein fractions, which were apparently specific for the respective protein, showed that all myeloma proteins (with the exception of a single gamma myeloma) were brought down by antisera prepared against normal gamma protein. The proteins of γ -myeloma showed stronger reactions than those of β -myeloma when the precipitation was performed with antisera prepared against various preparations of normal human γ -globulin as well as against rabbit γ -globulin. With Ouchterlony's agar method, it was demonstrated that heterogeneity was rare. It was present, however, in 2 cases, and in these there was a combination of β - and γ -globulins. These investigations demonstrate that each myeloma serum contains an increased amount of a specific protein. It is not yet known whether the pathologically increased protein present in myeloma really is a pathologic protein (i. e., one not present in normal plasma) or a normal protein, whose amount is increased. The hypothesis was made, however, that the myeloma proteins, even if not normal, are still related to normal protein. For a discussion of immunoelectrophoresis in myeloma sera see Grabar and Burtin and Heremans [81].

Another method of characterizing proteins is the determination of their amino acid content following hydrolysis. Such studies have been made in myeloma by a number of authors [118, 119], and have shown that the amino acid pattern is basically the same in normal γ -globulin and in myeloma globulin, although some individual differences exist [139]. Putnam's studies [140] on the N-terminal amino acids form an excellent basis for the discussion of this problem. Fingerprinting according to Ingram [141] will probably solve many problems.

Thus, we know little of the real nature of the pathologically increased globulin fractions found in myeloma. Tests of the serologic reactions of myeloma sera sometimes show an increased anticomplementary titer, resulting in inhibition of the Wassermann reaction. On the other hand, one seldom finds any other serologic phenomena such as those often seen in hyperglobulinemia with broad gamma fractions.

Heterophile antibodies, sheep cell agglutinins, and antistreptolysins are often found when the γ -fraction is broader than normal but very rarely in myeloma sera. It is possible that this difference is due to the fact that in myeloma only one or very few of the γ -fraction components are pathologically increased [103]. This concept is consistent with the narrow, high electrophoretic peak seen in myeloma, which is of great diagnostic importance, and with the ideas of Burnet. I have called such increases in one or a few γ -fractions "monoclonal"; i. e., deriving from one clone of plasma cells.

Bence Jones proteinuria is well known in myeloma, and for a long time Bence-Jones protein (BJP) was considered the most important single diagnostic finding in myeloma. The protein has unusual properties, is easy to demonstrate, and allows the rapid diagnosis of an unusual disorder. More recent studies have suggested that the criteria for the demonstration of a BJP are probably to be taken less seriously than

previously believed. On the other hand, the presence of true BJP is virtually pathognomonic of myeloma, although certain other diseases, such as lymphatic leukemia, may rarely produce BJP. BJP has also been found in cancer metastases to bone, but this finding is yet to be generally accepted. The properties of BJP during heat, boiling, and cooling are well known. It should be mentioned, however, that electrophoresis of urine plays an important role in the identification of pathological proteins in the urine. The frequency of Bence-Jones proteinuria is reported differently by different authors. Kahler's triad of 1889 was considered pathognomonic for 40 years: bone pain, deformity, albuminuria. Today, the diagnostic signs are found by bone x-ray, serum protein pattern, and sternal puncture. It is still believed by many, although falsely, that BJP is a frequent initial finding in myeloma. In 13 consecutive cases observed by the author [142], only 3 showed Bence-Jones proteinuria initially. It is quite clear that the absence of this finding is of little diagnostic importance.

BJP comprises a group of low-molecular proteins in which the individual proteins are not all alike. BJP are not products of γ -globulin breakdown. Their synthesis occurs independently of the formation of the pathological serum globulin, as shown by Hardy and Putnam [143].

Macroglobulinemia and Cryoglobulinemia

Large amounts of globulin cause increased viscosity of the serum. This increase is proportional to the content of globulin as, for example, in the usual hypergamma-globulinemias. Certain pathological globulins, however, cause an increase in viscosity which is disproportionate to their concentration [145]. It is a special characteristic of many such globulins that the increase in viscosity is abnormally large when there is a reduction in temperature. Waldenström suggested a temperature-viscosity index to characterize such proteins, using the following proportion:

$$\frac{100 \times \text{relative viscosity at } 13^{\circ} \text{ C.}}{\text{relative viscosity at } 37^{\circ} \text{ C.}}$$

Values above 120 are pathological. Of especial importance in this respect are the so-called "cryoglobulins." By definition, a cryoglobulin must precipitate or gel on cooling.

The clinical syndrome of cryoglobulinemia was first described independently by Lerner and Watson in the United States [146, 147] and Flemberg and Lehmann in Sweden [148]. Certain globulins are precipitated from the plasma on cooling. This precipitation results in a blockage of small blood vessels by protein masses, and the course of the illness can be explained on this basis. When the body is cooled, the first thing to occur is edema of the skin, followed by erythema and, often, residual purpura ("cryoglobulinemic purpura"). At times, during cooling, one sees acrocyanosis, which is similar to that seen when patients with cold agglutinins are exposed to the cold. These seem to be the two outstanding examples of well-defined chemical reactions causing pathological symptoms related to low temperature. However, there are patients with marked increase of cryoglobulins in whom no symptoms of peripheral vascular disturbance occur. In one such recent patient, cryoglobulinemia was without clinical manifestation. There is then no clinical disease and the metabolic disturbance of globulin is designated "essential." It is probable that many of these cases belong to the group of collagen disorders. Recent observations of cryoglobulinemia in lupus

erythremias are of importance. Precipitation of cryoglobulins has also been observed in lymphocytic leukemia [150], myeloma, and lymphosarcoma [149].

In one patient, crystallization of a serum globulin fraction was noted not on exposure to cold but after evaporation of carbon dioxide at 37° C [152] in an open (not closed) container. Precipitates could be seen in the cornea of the patient, apparently because carbon dioxide is liberated here [153]. These corneal precipitates increased and the patient's vision became markedly impaired. The clinical syndrome was one of severe collagen disease with changes in the joints. Such cases of "false cryoglobulinemia" are exceedingly rare, but the possibility must be considered in every case of supposed cryoglobulinemia. True cryoglobulins should always redissolve on warming.

Sometimes gelation occurs following cooling, and the serum may then have various appearances [151, 11]. There may be gradual increase of viscosity to a very high degree, or one may see the sudden appearance, at some critical temperature, of a true gel. Sometimes, in addition, the serum may separate into two layers, the upper thin and liquid, and the lower thick and viscous. The precipitation of solid globulin has already been discussed. Various manifestations of gel formation may be seen at different times in the same patient, and they therefore must have the same explanation and are of no value for differential diagnosis [11].

At this point, we may discuss a phenomenon which sometimes causes practical difficulties in the clinical laboratory. In myeloma and in other diseases with disturbed serum proteins, clumping of the erythrocytes may occur and make it impossible to do a red cell count. There are three possible explanations of this phenomenon:

(1) The occurrence of a cryoglobulin, which precipitates in the cold and causes irregular distribution of the erythrocytes; such precipitation does not occur at 37° C, and this situation somewhat resembles the cold agglutination which is seen in, for example, atypical pneumonia.

(2) The occurrence of a globulin factor which is precipitated by mercury salts (Hayem's solution); in such cases, the use of physiological salt solution as the diluent, instead of Hayem's solution, prevents the clumping of the red cells in the chamber.

(3) The occurrence of cold agglutination. Counting of the red cells at 37° abolishes this phenomenon.

A property of highly viscous sera is often a strong *euglobulin reaction*: i. e., flocculation on dilution with water (Sia test) or on dialysis against water [154]. When these globulin fractions are examined in the ultracentrifuge, they often show a very high sedimentation constant (18–20 S), suggesting that they belong to the group known as macroglobulins. It is easy to understand that a large molecule with a molecular weight of about 1 million will not remain in solution except under very favorable conditions.

Macroglobulins are especially seen in the disorder known as "*macroglobulinemia*" [145, 154]. The serum contains a globulin which, on electrophoresis, migrates with the gamma globulin or the β_2 -globulin, or between these two. The electrophoretic spike is always narrow and high, as seen in systemic disorders (myeloma), and not wide, as seen in secondary hypergammaglobulinemias (collagen disorders, hepatic cirrhosis, lymphogranuloma venereum, etc.). The disease macroglobulinemia is characterized by normochromic anemia, which may be quite severe in late stages; a normal leukocyte count with a tendency to lymphocytosis; and a bone marrow infiltrated with small lymphocytic cells, some plasma cells and sometimes also tissue mast cells [155]. The serum albumin is usually markedly reduced and there is a tremendous increase in pathological globulins. The total serum protein may be as high as 12 g/100 cc. with

7 g of globulin. Edema is often present, as well as a hemorrhagic diathesis with mucosal bleeding from the nose and mouth and bleeding into the ocular fundi. Purpura, however, is rare. There may be excessive bleeding after cuts and injuries, and the bleeding and coagulation times may be prolonged. The hemorrhagic diathesis is probably a physico-chemical effect of the highly viscous macromolecular globulin adhering to the platelet surface.

The diagnosis can usually be made by marrow puncture. Colored photographs of the bone marrow are found in [154]. There are, however, many cases in which the bone marrow aspiration is normal. The disorder differs clinically from myeloma by the absence of skeletal changes and skeletal pain. It can be distinguished from lymphatic leukemia by the absence of a leukemic blood picture and the lack of marked lymphadenopathy, although palpable lymph nodes may be present and hepatosplenomegaly is common. The anemia is progressive. The patients die with severe cachexia, often with marked edema, which may perhaps be due to the hypoalbuminemia. The course of the disease may be very protracted. Diagnosis is aided by the precipitation of euglobulin with distilled water and the abnormally marked increase of serum viscosity at low temperature. The results of ultracentrifugation are diagnostic. Serologic studies [156] and amino acid analyses have been made in cases of macroglobulinemia by various authors [157]. Newer methods for the diagnosis of macroglobulinemia are detailed by Heremans [81].

Problems of Protein Metabolism.

Perhaps the most important current problem in the investigation of proteins, both clinically and theoretically, is the *investigation of the regulation of protein synthesis*. Recent studies have shown that desoxyribosenucleic acid (DNA) acts as an indirect template for protein synthesis, but the exact mechanism is not known. Most authors are of the opinion that the hereditary unit, "the gene," is composed of DNA, probably with a coating of protein. On the basis of studies of inborn errors of metabolism, it has been suggested that many chemical mutations are the result of a defect in a specific enzyme. This hypothesis has been based chiefly on studies of the mold, *Neurospora*, which shows frequent mutations. According to this view, metabolism is considered as an expression of an enzyme mosaic, which in turn is determined by a gene mosaic. All enzymes are proteins and this hypothesis is of course also valid for the synthesis of proteins generally. The final synthesis of the entire group of γ -globulins is probably determined by a single gene. When this function of the gene is deficient (there is a "malformed" template), gamma globulin is not synthesized. It is easy to understand that the synthesis of a specific, highly differentiated substance such as an enzyme depends on the presence of the proper template; it is more difficult to grasp how a single gene can regulate the synthesis of a large proportion of the plasma proteins: e. g., the γ -globulins or the albumins.

Can these ideas be applied to the synthesis of the pathological *plasma proteins*? Few hereditary dysproteinemias have as yet been studied.

It is evident that family studies will do much to clarify the problem of causal genetic relations between different types of protein disturbances. The reader interested in these problems will find a discussion in a recent review [130]. Curiously enough, both agammaglobulinemia occurring in males from infancy and so-called "acquired" hypogammaglobulinemia in females may have a genetic basis; the first

instance sex linked, and the second, autosomal recessive (Wollheim [159]). An interesting family has been published by Lindholm (in Swedish) in which a man who developed symptoms of hypogammaglobulinemia had several relatives with narrow-banded hypergammaglobulinemia, among these one paternal uncle with myeloma. We have seen probable myeloma (monoclonal, M type hypergamma) in one woman whose sister had typical L. E. D. (polyclonal, broad hypergamma with several positive serological reactions). It therefore seems probable that not only a tendency towards hypo- or hypergammaglobulinemia but also a certain "instability" of the γ -forming system may occur in families.

It is possible that several illnesses, whose most prominent manifestations occur in the serum protein picture are hereditary. *Myeloma* remains the most remarkable disorder in this regard. The author investigated this problem in the region of Uppsala, where many cases of myeloma occur. Attempts to demonstrate heredity were unsuccessful; however, this is not absolutely conclusive, if the tendency to myeloma were recessive, as the disease always occurs quite late in life. Some instances of macroglobulinemia occurring in relatives are known.

Of greater importance to the clinician is the question as to whether there are any findings in the many cases of *unexplained hyperglobulinemia* which suggest a hereditary pattern. We have studied a family in which an elevated sedimentation rate was at first thought to be due to essential hypergammaglobulinemia. Three members of this family subsequently showed transient findings of disseminated lupus. The clinical diagnosis of lupus was therefore made, even during asymptomatic periods. Dameshek's comparison with an iceberg is appropriate here: only a small part of the disease is clinically visible, while the fundamental, most important part is hidden. An increase in gamma globulin and a positive Rose Waaler test may then be the only indication of disease [105, 106, 159].

In my opinion, it is likely that many of the disturbances of the blood proteins which we still understand poorly are in truth "essential"; i. e., the altered protein synthesis is indeed the cause of the entire syndrome. Slight anemia and increase of the sedimentation rate are obvious, but still other manifestations will come "to the surface." The basic cause of the disease is the disturbed synthesis of protein; eventually, this may help explain the so-called hyperimmune response in the collagen disorders.

The clinical study of blood proteins will be aided by our increasing understanding of the protein-building templates.

Disturbances of Amino Acid Metabolism

There are many different types of the so-called inborn, hereditary disturbances of amino acid metabolism. In the typical intermediate metabolic disturbances, there is impairment of transformation of substance A into the next metabolic substance B. These disturbances may be illustrated by the absence of an enzyme AB. Examples of such disturbances are alkaptonuria, one type of cystinuria (page 448), and the so-called Fölling's disease, in which there is a hereditary tendency to excrete phenylpyruvic acid in the urine. Tyrosinosis may possibly be a similar disorder. The basis for those cases of cystinuria that are now known as Fanconi syndrome is quite different. Here, there is a hereditary cystinuria, together with glycosuria, phosphaturia, and generalized aminoaciduria. Recent studies by Dent suggest that this is a primary disorder of the kidneys. In Wilson's disease (page 417) there is also aminoaciduria.

In galactosemia there is also excretion of amino acids in the urine probably caused by competition between galactose and these substances in the renal tubule.

The classical hereditary disturbance of amino acid metabolism is *alkaptonuria*, which was described in the 1880's. This disorder must be thought of when an alkaline urine darkens on exposure to air. The presence of large amounts of homogentisic acid is the cause of this phenomenon. Alkaptonuric urine reduces like diabetic urine, but yeast does not influence the reducing activity. For many decades, the excretion of homogentisic acid is the only finding, but after the age of 40 or 50 the patient often develops a chronic disease of the joints which resembles osteoarthritis. There is apparently deposition of pigment in the cartilage, with the development of "ochronosis," in which all the bones become dark brown to black. The nature of the biochemical disturbance is discussed on page 447 (Fig. 80). The hereditary nature of the disease is well established; as in some other inborn errors of metabolism, transmittance may change. There is a recessive (more common) form, and a rare dominant form [160]. The disease itself is very rare.

More common is another intermediate disturbance of amino acid metabolism, first described by Fölling in Norway as "*imbecillitas phenylpyruvica*" [161]. It is usually discovered accidentally when the urine is tested for acetaldehyde by Gerhardt's method, and is found to turn green. The first patients described by Fölling were mentally defective. He was soon able to demonstrate that the metabolic disturbance resulted in a clinical disease. Pigment formation is defective, so that the affected children are very pale and often have light hair. There is distinct hypotonia of the muscles. Physicians who have seen many such cases state that they have a distinctive odor. Mental development is defective. Psychic disturbances are greater in some patients than in others; in most, there are severe manifestations of idiocy. In contrast, there is no impairment of physical development.

Various theories were suggested to explain this disorder. Certain authors, e. g. Bickel [162], believed that the increased phenylalanine in the blood was directly toxic to the brain, and attempts were made to give a diet poor in phenylalanine for therapeutic purposes, with unquestionable success. It is of great interest that correction of a metabolic defect should benefit the mental function of these children. In recent reports many other metabolites have been described in the urine of these patients.

This disorder is apparently recessive. Consanguineous marriages are more common in the parents of these patients than in the normal population. The number of afflicted siblings also agrees with the concept of homozygosity. The disorder is not very rare: in England and in the U. S. A. it was found that the incidence was 1 case in 25,000 individuals. The incidence of heterozygotes would then be 1:500.

A third, very rare, disturbance of tyrosine metabolism was described in 1932 by Medes [163]. This "*tyrosinosis*" occurred together with myasthenia gravis. The urine contained reducing substances, but the reducing substance was not glucose but parahydroxy-phenylpyruvic acid, which was present in large amounts. There is apparently an absence of an enzyme function for the oxidation of tyrosine to homogentisic acid (page 447), analogous to the situation in alkaptonuria, where there is impaired destruction of homogentisic acid. Medes' case is the only one reported in the literature to date, but was very thoroughly studied by her.

"*Albinism*" may be included among the disorders of amino acid metabolism, although it is not yet known for certain whether there is a disturbance of tyrosine metabolism in this disorder. In albinism, there is a lack of the normal pigment in the skin, hair, and eyes. The normal skin pigment, melanin, is synthesized in the pres-

case of the so-called "dopaoxidase," which oxidizes a chromogen apparently related to dihydroxyphenylalanine (page 398). Albinism is hereditary and the patients are almost all homozygous; their parents often show consanguinity. A dominant inheritance is, however, also seen. Occurrence in identical twins has been described. The disturbance is not uncommon. It has an incidence of approximately 1:10,000.

It is perhaps strange that so many intermediate disturbances in tyrosine metabolism are genetically determined. Fig. 80 summarizes these chemical malformations (see also p. 415, Fig. 73).

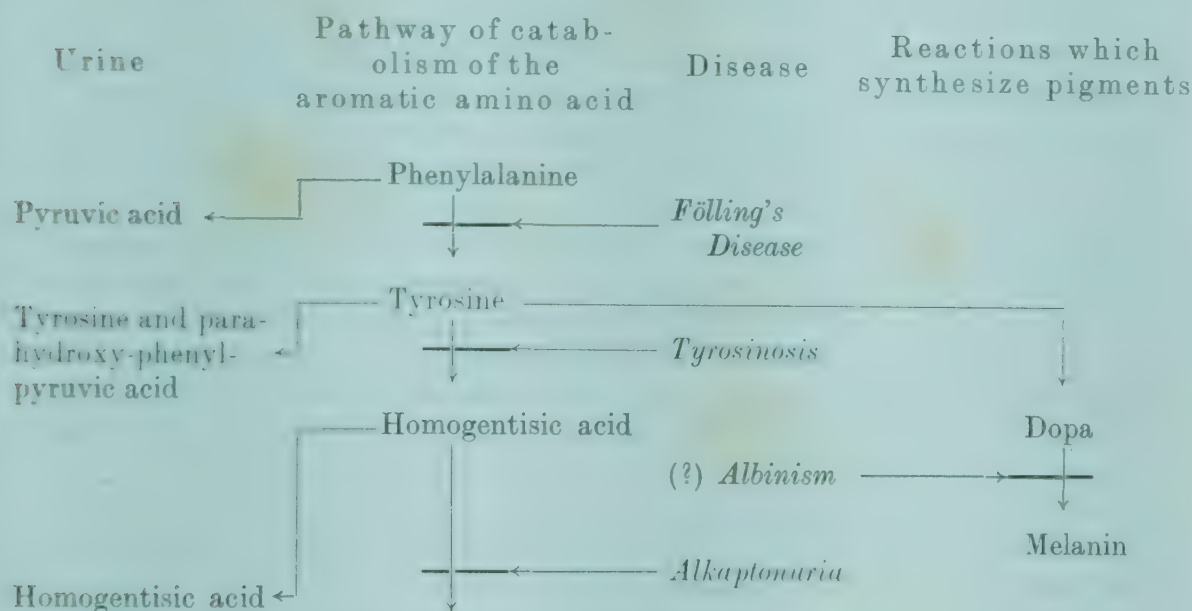


Fig. 80. Schematic representation of disturbances of tyrosine metabolism.

Similar disturbances in the metabolism of other amino acids, such as tryptophane, leucine, or histidine, have not been described until recently. On the other hand, there is a type of cystine diathesis which is probably not merely a disorder of the kidneys, but is also a metabolic disturbance [169]. There are still other recently discovered disturbances such as maple-syrup disease and β -amino-isobutyric aciduria with abnormal metabolites in the urine. Other conditions which are probably inborn errors of metabolism include pyridoxine deficiency and orotic aciduria with hematological symptoms.

The question as to why life is still possible despite the blocking of a metabolic pathway, can be answered by the hypothesis that there is only a partial disturbance of the synthesis of the enzyme in question (malformation).

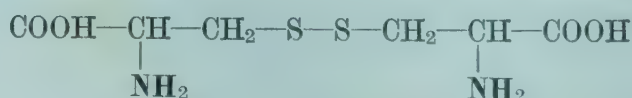
Another group consists of disturbances in which there is a primary defect in tubular reabsorption of certain compounds. It is probable that a lack of certain substances (cf. intrinsic factor), "pilots," might be the explanation.

The *cystinurias* have been intensively investigated, especially by Dent and Harris [165, 166]. It had previously been believed that the presence of cystinuria always connoted an "internal" disturbance of amino acid metabolism, as in alkaptonuria and in phenylketonuria. Recent polarographic and microbiologic methods have allowed the exact determination of cystine in the blood. In cases of "cystinuria," there is not necessarily an accumulation of cystine in the blood. In Dent's cases, the blood cystine

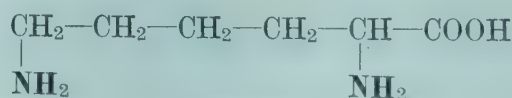
level was usually somewhat reduced. There are, however, certain cases, especially in children, in whom there is a tremendous deposition of cystine in the body ("cystinosis"), which cannot be due to a renal cause. It is therefore probable that there are several different mechanisms which result in the various disturbances of cystine metabolism. Dent and Harris separated 2 clinical entities: (1) congenital cystinuria of children; and (2) classical cystinuria. In the apparently congenital cystinuria described in children as the *Fanconi-Lignac syndrome* [143], the urine also contains other amino acids, glucose, and phosphate. This must be due to the same type of disturbance of renal tubular reabsorption as is seen in renal glycosuria or in renal hypophosphatemia, except that the disturbance affects more than just glucose or phosphate reabsorption. The glycosuria is of no clinical importance, but the chronic hypophosphatemia leads to marked changes in the skeletal system with osteoporosis and fractures, which at one time were called renal rickets, and dwarfism is present. Paper chromatography of the urine shows that this condition is one, not of isolated cystinuria, but of aminoaciduria. There are no cystine stones.

Fanconi's syndrome is often seen in siblings, but never in successive generations. It is believed that this rare disorder is a simple, recessive one. Very likely the inherited abnormality is one of disturbed renal morphology. Thus, microdissection of the nephrons in this disorder by Darmady and co-workers showed that the first portion of the convoluted tubules was atrophied and looked "like the neck of a swan." This is the section of the renal tubules which normally has to do with reabsorption of phosphate, glucose, and amino acids.

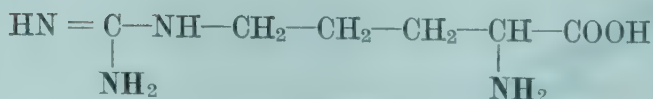
The chief finding in *classical cystinuria* on the other hand, is the occurrence of cystine crystals in the urinary sediment, and often also nephrolithiasis, with the stones formed of cystine. In addition to cystine, the urine contains increased amounts of the diamino acids arginine and lysine. Serious complications include kidney infection and uremia. The disorder is apparently hereditary; the cystinuria is accompanied by lysinuria and argininuria, but not by the excretion of any other amino acids in the urine. Dent therefore suggested the hypothesis that aliphatic substances with 2 amino groups separated by 4 to 5 carbon atoms are reabsorbed by the same mechanism (although not necessarily at the same site), and that a disturbance of this reabsorptive mechanism affects all three diamino acids, so that they all appear in the urine together.



Cystine



Lysine



Arginine

The inheritance of cystinuria with concomitant lysinuria and argininuria was thoroughly investigated by Dent and Harris. They found a very complicated mechanism, so that the problem is not yet solved. It is possible that patients who show

severe cystinuria are homozygous, and those with mild cystinuria are heterozygous. This condition has also been observed in identical twins.

Wilson's disease also shows a disturbance of tubular reabsorption of amino acids (page 417). In this disorder, the amino acids appear in the urine in about the same ratios as they are present in the blood. Polypeptides with a terminal amino-dicarboxylic acid are said to appear in the urine. It is possible that the constant loss of amino acids (and polypeptides?) leads to secondary damage to the liver. The relationship of the hepatic cirrhosis to the basal ganglia lesions is still unexplained. Asymptomatic relatives of patients with Wilson's disease may show excretion of amino acids in the urine, and may therefore be considered to have latent forms of the disorder.

Galactosemia. Still another type of hereditary aminoaciduria has recently been described, which occurs in galactosemia. In galactosemia of children, there is apparently a wide-spread absence of the enzyme necessary to convert galactose from lactose into glucose. If the diagnosis is made, so that carbohydrate feeding with glucose may be substituted for feeding of milk, the children may develop normally. Aminoaciduria has been found in this disease: the increased excretion of galactose in the glomerular filtrate probably prevents normal reabsorption of the amino acids by the tubules. This is a further example of the interrelationships of various enzyme defects and tubular reabsorption mechanisms as regards metabolic processes necessary for normal life. The heredity of galactosuria is apparently recessive. Correct diagnosis and a proper regimen will probably save these children in the future until they can lead a "milkless" life of an adult.

Two extremely interesting conditions have recently been discovered by Dent and his group. The first, called Hartnup disease [164], leads to pellagra like symptoms plus ataxia and is apparently caused by a disturbance of tryptophane absorption and excretion on a hereditary basis. Cases have been reported from England, Holland, and Germany. The other, which is still rarer, causes epilepsy, ataxia, and mental retardation, and is characterized by the excretion of succinyl-arginine in the urine. It has been described in two siblings.

The importance of such studies for a correct understanding of hereditary mental disorders is evident and considerable progress may be expected along these lines.

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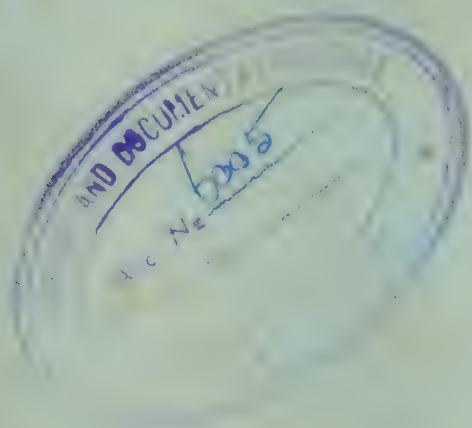
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